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PRELIMINARY OBSERVATIONS ON THE INSECT PESTS OF DRY FRUITS, OAT MEAL AND ARMY BISCUITS AND THEIR CONTROL

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India imports large quantities of dry fruits, oat meal and army biscuits annually. Insect pests attack and spoil these articles during storage with the stockist and the dealers. We have seen several tinfuls of oat meal reduced to foul-looking, worthless mass of dry powder and dry dates webbed together into an evil-looking and evil-smelling mass. There was no demand for a mass supply of these articles and the damage done by insect despoilers of our dry fruits, oat meal and army biscuits went unnoticed with the result that they did not attract the serious attention of Indian Entomologists and as such our knowledge about them remained very meagre indeed. The present war, however, has necessitated re-orientation of our attitude towards these pests and has forced on us the need for protecting dry fruits, wheat products, etc. from their unwelcome attention. Our brave soldier and our civilian population must have wholesome and clean food which is free from insect pests. Therefore work on the insect pests of dried fruits, oat meal and army biscuits was undertaken in the beginning of 1942. These researches have to-date brought to light 15 different kinds of insect pests in the Punjab.

The statement given below shows the various insects collected in the Punjab from various food grains, food products, dry fruits, etc.

It is our experience that these insect pests are in no way less destructive than the pests of stored grains (Rahman, 1942) and as such it is imperative that researches on them on an all-India basis should be undertaken forthwith in order to study their habits and behaviour thoroughly with the object of evolving satisfactory and easily applicable methods of combating them.

No.	Name	Family	Food
1	*Sivanus surinamensis* L. ..	Cucujidæ	Raisins, dry dates, all kinds of dry fruits, etc., cereal grains, biscuits, suji (semolina), vegetable seeds
2	*Læmophlæus minutus* (*pusillus*) Schon	do.	Wheat, jowar, biscuits and other products of wheat
3	*Tribollum castaneum* Hbst. ..	Tenebrionidæ	Cereal grains, dry fruits, biscuits, compressed wheat and oats
4	*Tribolium confusum* Duval ..	do.	Turmeric
5	*Attagenus piceus* Olivier ..	Dermestidæ	Raisins, zira, ajwain, carpets and woollen materials
6	*Carpophylus hemipterous* Linn. ..	Nitidulidæ	Dried and decaying fruits
7	*C. dimidiatus var mutilatus* ..	do.	Decaying pomegranate and dry fruits
8	*C. dimidiatus* F.	do.	Decaying bæl fruit.
9	*Tenebrioides mauritanicus* L. ..	Trogositidæ	Stored grains, dry fruits
10	*Lasioderma sericornæ* Fab. ..	Anobiidæ	Stored tobacco, poppy, chillies, dry fruits
11	*Sitodrepa* (*stegobium*) *panicea* L.	do.	Coriander seeds, raisins and many other things
12	*Stathmopoda trissorrhiza* M. ..	Heliodinidæ	Dry fruits, etc.
13	*Ephestia cautella* Wlk.	Phyctidæ	do.
14	*Corcyra cephalonica* Stn. ..	Pyralidæ	Cereal grains (rice, wheat, oats, barley) and dry fruits
15	*Sitotroga cerealella* Ol. ..	Gelechidæ	Compressed white oats, wheat, jowar seeds and chillies

I. Important pests

(1) *Oryzæphilus* (*Silvanus*) *surinamensis* L. : *Cucujidæ-Coleoptera* (Saw-toothed grain beetle)-Food.—This is a cosmopolitan insect of omnivorous habit which has proved itself a serious and highly destructive pest (Rahman, 1942) of such dry fruits as raisins, dried dates, pistiacho, walnuts, and such wheat products as ' suji ' (semolina) and biscuits; we also found it destroying garlic, and seeds of sesame melons, water melons and gourd, etc. Elsewhere it has been reported doing damage to dried cherries, dried apples, dried pears, dried prunes, cacao, cocoanut and chocolate.

Habits and life-history.—Adults are negatively phototropic: they are always found at the bottom of the contents of a container. When

exposed, they at once bury themselves under their food. Because of their retiring and shy nature, their copulation could not be studied.

The Saw-toothed grain beetle breeds from April to November. According to Back and Cotton (Patton, 1930) it lays eggs in small batches and deposits them out of sight in cracks and crevices as well as singly in finely ground foods, while according to Rahman (1942) it lays eggs in or near its food. In our breeding experiments we found a female to lay eggs singly in tunnels which it bored in the pericarp of a dried fruit. A female was found to lay 46–185 eggs in its life time. The eggs hatched out from 7 to 14 days. The grub came out of the egg through the micropylar end by pushing off the operculum. After hatching they bored in the pericarp of dried fruit where they fed. They were full-grown in 32–39 days. The grubs avoided light and completed their growth concealed in their tunnels. They pupated in the fruit and the pupal stage occupied 13–20 days. The life-cycle of the pest was thus completed in 54–73 days. Table I gives the duration of its life-cycle during June-October.

TABLE I

Showing the duration of the life-cycle of the Saw-toothed grain beetle during June-October

Date of oviposition	Date of hatching	Date of pupation	Date of emergence	Duration in days of			
				Egg stage	Grub stage	Pupal stage	Life cycle
18–6–1942	25–6—1942	27–7—1942	11–8—1942	7	32	15	54
18–6–1942	26–6—1942	28–7—1942	12–8—1942	8	32	15	55
7–7–1942	14–7—1942	16–8—1942	31–8—1942	7	33	15	55
13–7–1942	21–7—1942	25–8—1942	7–9—1942	8	35	13	56
15–8–1942	26–8—1942	31–9—1942	15–10–1942	11	36	15	62
15–8–1942	24–8—1942	30–9—1942	15–10–1942	9	37	16	62
5–9–1942	17–9—1942	20–10–1942	6–11–1942	12	33	17	62
20–9–1942	4–10–1942	12–11–1942	1–12–1942	14	39	20	73

(2) *Attagenus piceus* (*Dermestidæ Coleoptera*).—This species is a well-known pest of carpets and woollen goods but we found it seriously attacking raisins, dry dates, 'ajwain' (*Ptychotis ajwain* D.C.: lovage) and black "zira" *Carum carni* L.: carryaway.

The grub destroys dry fruits ,"zira" and "ajwain" not only by its actual feeding but also by mixing up excreta and cast skins with the attacked fruit.

The grubs avoid light and when exposed to it they at once hide themselves. Like Khapra (Rahman, 1942) they also cling to rough surfaces.

To take advantage of this habit of the pest, the infested material should be spread on a sheet of paper and after the entire lot of grubs have clung to it, the material should be shaken off to another sheet.

(3) *Stathmopoda trissorrhiza* Meyr. (*Heliodinidæ*: *Lipidoptera*) (Raisin web moths).—This moth was collected for the first time in October, 1942 at Lyallpur from raisins and dried dates doing serious damage to them.

Raisin web moth hibernated as a caterpillar during November–March. It became active in April and remained so upto October. The female laid eggs singly and attached them to the fruit. The caterpillars are pale whitish in colour with brownish head. Their attack always started at the bottom of the stored fruits, but as it progressed, the upper layers also were attacked, the whole of the fruit becoming a single mass united by silken webbings and strewn with excreta. When full-fed the caterpillars pupated in whitish silken cocoons.

(4) *Ephestia cautella* WLK. (*Phycitidæ*: *Lepidoptera*) (Fig moth).—We collected it from Rawalpindi from dry apricots in March, 1943 and from stored apples at Kulu in January, 1936.

Females attached their eggs loosely in grooves and wrinkles of dry fruit. The larvæ on hatching fed on the skin of fruits, but later on they bored into them. The caterpillar is pinkish with a brown head. Its body has a broad transverse stripe just behind the head and rows of prominent dots on the back. When full-fed the caterpillars leave the fruit and spin up a thin silken cocoon, in the interspaces of infested fruit or on the sides of the container, in which they pupate. The pest completed its life cycle in about a month. It remained active from April to October.

(5) *Corcyra cephalonica* Staint (*Pyralidæ*: *Lepidoptera*) (Rice moth).—In addition to cereal grains mentioned by Rahman, (1942) we have found this pest damaging raisins at Lyallpur. Adults mated soon after emergence, mating lasting for 3–8 minutes. Immediately after copulation females laid eggs, generally singly, occasionally 3–4 together. A single female was found to lay a maximum of 115 eggs in its life-time. Egg stage occupied 6–7 days. The larval stage was completed in 20–28 days. Pupation took place in silken tunnels. Pupal stage was completed in 10 days.

(6) *Sitotroga cerealella* Oliv. (Gelechidæ: Lepidoptera). (Grain moth).—In addition to the food mentioned by Rahman (1942), we have observed this insect infesting chillies seriously (*Capsicum anum*) at Kulu and compressed white oats (manufactured by an Indian firm) in tins. In the latter case the contents were found to be reduced to whitish powder

absolutely unfit for human consumption. The pest is active during summer but hibernates as a larva in winter. In summer its life cycle is completed in 35 to 39 days, egg stage 4 to 6 days, larval stage 21 days and pupal stage 10 days.

II. Control

Fumigation of the infested stored products with Hydrocyanic acid gas and Carbon bisulphide is considered a panacea for all insect ills. But these poisons cannot be used with immunity and safety by all those interested in the control of these pests. Therefore, simpler, more easily and generally applicable methods of combating them are required and in order to evolve such methods (as also to stimulate further research in this point) we tested various methods of packing various gases and heat as means of preventing or controlling insect damage. The results of these investigations are discussed below :—

(1) *Packing.*—Such articles of diet as Quaker oats, Army biscuits, etc., are sold packed in containers. If proper care is not exercised in preventing insects from getting access to the container then, they offer ideal conditions for multiplication of, and damage by, insects.

The container may be made of tin or the article may be wrapped in waxy tissue and the packet contained in a hard card-board case wrapped again with celluloid paper. Tin packing is however much superior to card-board packing.

(2) *Treatment with gases* : *Technique.*—Wide-mouthed glass bottle of 1000 c.c. capacity fitted with a cork having two bores were employed in these experiments. A long glass tube was inserted in one of the bores so that one end of the tube reached the bottom of the bottle. The other end was connected with a rubber tube, and gas was discharged in it. The second bore of the cork was fitted with a small glass tube which acted as an outlet for the air from the bottle. After filling the bottle with the gas the cork with two bores was immediately replaced by a glass stopper after smearing it with a little vaseline to make it fit the mouth of the bottle tightly. The contents of the bottle were examined at the termination of the stipulated exposure. To study the mortality of the eggs the bottle after the stipulated exposure was opened and gas removed. It was again examined after 1–2 weeks to see if any eggs could hatch out. Various workers have tried hydrogen sulphide, sulphur dioxide and carbon dioxide with varying results, but we tried in addition to these gases oxygen, chlorine, ammonia and methane.

(*a*) *Oxygen.*—Bottles containing dry fruit infested with the various stages of the Saw-toothed grain beetle, Fig moth, Raisin web moth and

Red flour beetles were charged with the gas and examined after 24 and 48 hours exposure, but in no case any mortality was observed. Examination of the treated material after 2 weeks showed that their eggs hatched normally. Therefore, oxygen is useless for the disinfection of dry fruits against insect pests.

(*b*) *Chlorine.*—This gas proved fatal for the insects infecting dried fruits, oat meal, biscuits, etc., as all their stages perished when exposed to it for 24 hours. Raisins and dry dates, however, absorbed the gas to such an extent that they retained its smell for several months after treatment. The treated material was not freed of its smell even after exposure to sunlight. Further experiments are, therefore, needed to find out methods by which the treated material can be freed of its disagreeable odour before it can be recommended for use against insect pests of dry fruits, etc.

(*c*) *Ammonia.*—It killed all stages of insects after an exposure of 24 hours, but it imparted disagreeable odour to the treated material which could not be removed. It also spoiled the original flavour of the fruits.

(*d*) *Hydrogen sulphide.*—This gas killed cent per cent of the insect pests, but ruined the taste of the treated fruits and imparted an objectionable smell to them. Bad smell, however, was removed by exposing the treated fruits to sun heat for two hours, but taste remained unwholesome.

(*e*) *Carbon dioxide.*—The pests when exposed to this gas for 24–48 hours did not die, but cent per cent of them were killed when exposed to it for 96 hours. The gas did not harm the dry fruits in any way even after an exposure to it for several months. Thus if fruits are preserved in an atmosphere of carbon dioxide they will remain free from pests.

(*f*) *Sulphur dioxide.*—This gas killed all stages of the pests, but it imparted odour to the treated fruit. This odour was removed by spreading the treated fruits in sun-light for two hours. Sulphur dioxide, therefore, can be used for killing pests infesting dried fruits.

(*g*) *Methane* (*marsh gas*).—This gas killed all stages of the pests instantaneously and did not produce any ill-effects on fruits. It imparted a smell like that of chloroform to the fruit, but this smell was found to disappear by exposing the treated fruits to sun-light for two hours.

III. Heat

Heat is well known to kill insects. To utilise it for freeing dry fruits from insect pests, the following apparatus was devised after many experiments. This apparatus (Fig. 1) consists of two iron boxes, the inner for

holding fruit to be treated and the outer to hold water. The outer box and the inner box measure 2 × 2 × 2 feet and 1½ × 1½ × 1½ foot, respectively. The material to be treated is placed in the inner jacket and this jacket is placed in the outer jacket which is half filled with water. Both the boxes have small round holes in their lids, the central holes of the outer and the inner boxes are used for inserting a thermometer and the corner hole of the outer one serves as an escape for steam.

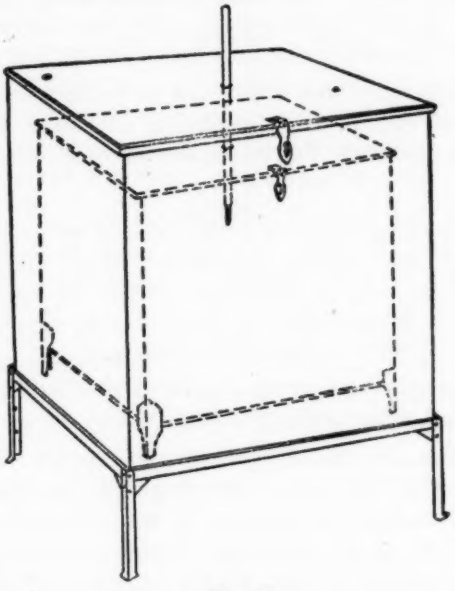

FIG. 1

Experiments carried out have shown that different insects have different lethal temperatures (see Table II).

TABLE II

Showing the lethal temperatures of pests of Dry fruits

No.	Pest	Temperature °C.	Humidity	Duration of exposure
1	The Fig Moth (*Ephestia cautella* Wlk.)	48	52	One hour
2	The Raisin Web Moth (*Stathmopoda trissorrhiza* Meyr.)	48	52	do.
3	The Rice Moth (*Corcyra cephalonica* S.)	48	52	do.
4	The Saw-toothed grain beetle (*Silvanus surinamensis* L.)	48	52	do.
5	The Red Flour beetle (*Tribolium castaneum* H.)	66	42	Two hours

Remarks.—(1) The time of exposure should be counted when the thermometer starts reading the stipulated temperature; (2) If the temperatures rises beyond the given limit it should be regulated by removing fire. The material is however not spoiled, even if the temperature rises to 90° C.

Indian Firms are taking up in these days the manufacture of food products like compressed wheat, oats, oat meals, etc. They are finding serious difficulties on account of the insect pests damaging their products which in consequence are rejected in the market. The manufacturers will find the following instructions useful for keeping their products free from insects:—

1. The main store of the grains should be kept free from pests by superheating before storage and fumigating afterwards with Hydrocyanic acid gas *vide* instructions in *The Indian Journal of Agricultural Science*, **12,** pp. 851–64.

2. Before packing, the article should be exposed to a temperature of 48 to 66° C. for one to two hours depending upon the type of the insect and packing immediately. Same treatment should be given after packing. Small firms may use an apparatus of the size given above, and large firms may employ steam heating to big sized containers.

3. Packing cases should preferably be of tin, hard cardboard cartons wrapped in celluloid papers being the next best.

4. Products can also be efficiently preserved in an atmosphere of CO_2 gas in tin containers. The gas is sold cheap in cylinders. It should be liberated in tins immediately after packing them with the material and the tin containers sealed. In this case heat treatment is not required.

Dealers of dry fruits can get rid of the insect infestation by subjecting the dry fruits to temperatures of 48 to 66° C. for one to two hours depending upon the type of the insect. If the dry fruits are full of dirt and debris, they should be washed clean with water and dried at these temperatures by steam plant and packed.

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HYDROXYLAMINE IN ROOT NODULES

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Hydroxylamine was postulated as an intermediate in nitrogen metabolism in the plant long before there was any experimental evidence to support the view. Mazé (1920) for example suggested the hypothesis that in photosynthesis hydroxylamine (formed from nitrate and ammonia) reacted with carbonic acid giving rise to glycolaldehyde and nitrous acid. The possibility of amino-acids being formed by reaction between nitrogen-free organic compounds and hydroxylamine was suggested by Baly and Stern (1923). Experimental evidence for the existence of hydroxylamine in plants and in symbiotic bacteria has however been obtained only recently. Blom (1931) detected traces of hydroxylamine in Azotobacter cultures while Lemoigne *et al.* (1935) and Endres (1935) were able to show that such cultures contained carboximes which on hydrolysis yielded free hydroxylamine. Lemoigne *et al.* (1937) claim to have found volatile compounds of hydroxylamine in the fresh leaves of higher plants. Virtanen *et al.* (1938) in their study on the excretion of nitrogenous material by root nodules isolated the oxime of oxalacetic acid as its copper salt. As aspartic acid was also found among the products of excretion, Virtanen suggested that amino-acid synthesis by symbiotic bacteria takes place according to the following reactions :

$$N_2 \rightarrow NH_2OH \rightarrow COOH.C(NOH).CH_2.COOH \rightarrow COOH\text{-}CH(NH_2).CH_2.COOH \rightarrow \text{other amino-acids}$$

(by transamination according to Braunstein and Kritzman, 1937). These conclusions are of far-reaching importance and require detailed substantiation. Since the fact of excretion of nitrogenous material by legume bacteria has itself been questioned (Wilson *et al.*, 1938; Bond, 1937), in the present investigation, root nodules growing on several species of leguminous plants have been examined for their content of free and combined hydroxylamine. For the quantitative estimation of hydroxylamine a highly specific colorimetric method* has been worked out based upon its conversion to nickel dimethylglyoxime. By condensation with diacetylmonoxime in the presence of a large excess of the latter

* A note on the method was published in *Current Science*, 1943, 12, 79.

and under suitable conditions of temperature and pH hydroxylamine is quantitatively converted into dimethylglyoxime which is readily estimated by the rose-red colour which it gives on treatment with oxidised nickel solution (Feigl, 1924). Hydroxylamine in concentrations of 0·0005 mg. nitrogen per ml. can be estimated by this procedure.

For application to plant material a trichloracetic acid extract of the latter is prepared, hydrolysed to liberate combined hydroxylamine if the latter is also to be determined and after neutralization treated with diacetylmonoxime. The dimethylglyoxime produced is extracted out with ether, the ether removed by evaporation, the residue taken up in alcohol and the dimethylglyoxime present estimated colorimetrically by means of nickel.

Applying this proceedure to root nodule material, no free hydroxylamine was found in the nodules of any of the several species examined. If however root nodule extracts were first submitted to a preliminary hydrolysis by keeping at 100° C. for 6 hours in 3N sulphuric acid, considerable quantities of hydroxylamine appeared. Similar results were obtained when combined hydroxylamine was determined by the method described by Blom (1926). These results are summarised in Table I.

TABLE I

Combined hydroxylamine in root modules

mg. hydroxylamine-N per 100 g. nodules

	Present method	Blom's method
1. *Phaseolus mungo* (Green gram)	7·595	7·789
2. *Vigna catjang* (Karamani)	7·084	7·075
3. *Phaseolus radiatus* (Black gram)	6·745	6·738
4. *Dolichos biflorus* (Horse gram)	4·751	4·746
5. *Pithecolobium saman* (Rain tree)	8·742	8·737
6. *Pongamia glabra* (Pungu)	9·177	9·165

Experimental

Reagents.—(*a*) Hydroxylamine standard, containing 0·01 mg. hydroxylamine-N in 1 ml. 4·953 mg. hydroxylamine hydrochloride dissolved in 100 ml. The solution is stable for over two weeks.

(*b*) Diacetylmonoxime in N/10 ammonia, 5·6 g. dissolved in 10 ml. of N ammonia and diluted with water to 100 ml.

(*c*) Oxidised nickel solution 0·4479 g. nickel sulphate ($NiSO_4 \cdot 6H_2O$) dissolved in 1 litre. For each estimation 10 ml. of the solution are treated

with saturated bromine water added drop by drop till a distinct excess is present. After allowing to stand for 15 minutes normal ammonia is added drop by drop till excess bromine is decolorized.

Procedure.—The test solution containing between 0·025 and 0·075 mg. hydroxylamine-N is neutralised to pH 7·2 by the addition of ammonia and then treated with 1 ml. of diacetylmonoxime solution (B). After allowing to stand for half-an-hour at room temperature, 10 ml. of the oxidised nickel solution (C) are added and mixed and after five minutes made up to 25 ml. The rose-red colour which develops is compared with a standard hydroxylamine solution (A), 5 ml. of which are subjected to the same treatment as the test solution.

Proportionality between colour and concentration.—Comparison of the colour developed with hydroxylamine solutions of different concentrations varying from 0·025 to 0·075 mg. N in 25 ml. showed that within this range there is reasonably good proportionality between colour and concentration, so that estimation can be made of a solution containing 0·001 mg. hydroxylamine-N per ml. with an error of less than 4%, the accuracy being higher with concentrations more closely approaching that of the standard (Table II). Concentrations down to 0·0005 mg. hydroxylamine-N can be determined using a standard of half the strength of the foregoing (Table III).

TABLE II

Standard : 0·05 mg. hydroxylamine-N

Standard and test solution made up to 25 ml.

Reading of standard : 20

mg. hydroxylamine-N in test solution	Readings calculated	Readings found	% error (−)
0·02	...	...	...
0·025	40·0	41·3	3·25
0·03	33·3	34·3	3·0
0·035	28·6	29·3	2·4
0·04	25·0	25·3	1·2
0·045	22·2	22·4	0·9
0·055	18·2	18·3	0·55
0·06	16·7	16·9	1·2
0·065	15·4	15·7	1·94
0·07	14·2	14·6	2·8
0·075	13·3	13·7	3·0

TABLE III

Standard : 0·025 mg. hydroxylamine-N
Standard and test solution made up to 25 ml.
Reading of standard : 20

mg. hydroxylamine-N in test solution	Readings calculated	Readings found	% error (−)
0·01	..	..	..
0·0125	40·0	41·7	4·25
0·015	33·3	34·5	3·6
0·0175	28·6	29·4	2·79
0·02	25·0	25·6	2·4
0·0225	22·2	22·5	1·39
0·0275	18·2	18·4	1·09
0·03	16·7	17·0	1·2
0·0325	15·4	15·8	2·6
0·035	14·2	14·7	3·52
0·0375	13·3	13·9	4·5

Effect of Temperature.—In the preparation of dimethylglyoxime the condensation between diacetylmonoxime and hydroxylamine has been carried out at various temperatures, *e.g.*, at 100° C. by Adams and Kamm (1918) and at 70° C. by Semon and Damerell (1925). Consequently in the earlier experiments the reaction mixture containing the test solution and diacetylmonoxime was placed in a boiling water-bath. It was found however that the reaction was incomplete at this temperature. The effect of temperature of condensation on the colour values obtained are shown in Table IV which summarizes the results of experiments in which the colour

TABLE IV

Standard : 0·05 mg. hydroxylamine-N
Condensation with diacetylmonoxime at room temperature for half-an-hour
Test solution : condensation with diacetylmonoxime at different temperatures
Standard and test solution made up to 25 ml.
Reading of standard : 20

mg. hydroxylamine-N in test solution	% Error at the various reaction temperatures					
	At room temperature for ½ hour	At 75° C. for ½ hour	At 75° C. for ½ hour	At 75° C. for 1 hour	At 100° C. for ½ hour	At 100° C. for ½ hour
0·02	3·5	4·0	4·75	5·0	5·5	6·8
0·03	3·0	3·6	4·5	4·8	5·0	6·0
0·035	2·79	2·9	3·5	3·87	4·9	5·2
0·04	2·0	2·2	2·4	3·2	3·6	4·4
0·045	0·9	1·35	1·78	2·25	2·2	4·1
0·055	0·55	1·1	1·1	1·64	2·7	4·4
0·06	1·2	2·0	2·39	2·99	3·2	5·1
0·065	1·94	2·9	3·2	3·8	4·5	5·8
0·07	3·2	3·5	3·5	4·2	4·9	6·3
0·075	3·7	4·0	4·5	4·8	5·3	6·3

produced at different reaction temperatures was compared with that at room temperature taken as standard. Colour intensity is always lower at the higher reaction temperatures.

Effect of Alkalinity.—In the usual preparation of dimethylglyoxime no special attention appears to have been paid to control the degree of alkalinity at which the reaction between diacetylmonoxime and hydroxylamine takes place. Using diacetylmonoxime dissolved in different concentrations of ammonia, it was found that the reaction proceeded best at pH 8·8 to 9 which was obtained by dissolving the diacetylmonoxime in N/10 ammonia under the conditions described.

Effect of quantity of diacetylmonoxime used.—Considereble excess of diacetylmonoxime is required to make the conversion of hydroxylamine to dimethylglyoxime complete. Experiments with varying quantities of diacetylmonoxime showed that it is advantageous to use a quantity of diacetylmonoxime 150 times of that required theoretically.

The values obtained in the experiments on the effect of alkalinity and concentration of diacetylmonoxime are omitted in the interests of economy of space.

Determination of Hydroxylamine in root nodules.—The nodules were obtained from trees and plants growing in ordinary garden soil. The excised nodules were washed clean, dried between filter-paper and a weighed amount (usually 10 g.) ground thoroughly in a granite mortar with 8 ml. of 50% trichloracetic acid and centrifuged after addition of 20 ml. of water. The residue was twice washed with 20 ml. water each, the supernatant and washings filtered free from suspended particles, brought to pH 7·2 with ammonia and made up to 100 ml.

To a 10 ml. aliquot of the solution, 1 ml. of a 1% solution of diacetylmonoxime was added, the reaction mixture allowed to stand at room temperature for half-an-hour, adjusted to pH 4·5 with dilute sulphuric acid and then extracted in a continuous extractor of the Kutscher-Steudel type for 4 hours with ether. The ether extract was evaporated to dryness on a water-bath. The residue was taken up in 10 ml. of 95% alcohol and made up to 25 ml. 5 ml. of the solution was used for colorimetric estimation 10 ml. of the oxidised nickel solution being added and the whole made up to 25 ml. With root nodule material treated in this manner no colour was obtained although hydroxylamine added to the trichlor-acetic acid extract gave nearly quantitative recovery.

Estimation of combined hydroxylamine in root nodules—Recovery of added hydroxylamine.—To 10 ml. of the trichloracetic acid extract obtained as before 10 ml. of 6N sulphuric acid was added and the solution boiled for 6 hours, filtered through kaolin and neutralised to pH 7·2 with ammonia. The solution was now treated in the same manner as previously described for the unhydrolysed extract. To another portion of the extract 1 mg. hydroxylamine was added and the estimation proceeded with as before.

In the earlier experiments, instead of using a continuous extractor, the estimations were carried out by shaking repeatedly with ether in a separating funnel; the results however were consistently lower than when a Kutsher-Steudel extractor was used. The results given in Tables V and VI are from

TABLE V

Without added hydroxylamine

mg. Hydroxylamine

	Before hydrolysis		After hydrolysis	
	Extn. by shaking	Extn. in cont. extr.	Extn. by shaking	Extn. in cont. extr.
Phaseolus mungo (Green gram) ..	0·00	0·00	17·79	17·91
Vigna catjang (Karamani)	0·00	0·00	16·58	16·73
Phaseolus radiatus (Black gram) ..	0·00	0·00	15·72	15·86
Dolichos biflorus (Horse gram) ..	0·00	0·00	12·29	12·43
Pithecolobium saman (Rain tree) ..	0·00	0·00	20·47	20·61
Pongamia glabra (Pungu)	0·00	0·00	21·47	21·61

TABLE VI

With one mg. added hydroxylamine

mg. Hydroxylamine

	Before hydrolysis		After hydrolysis		% Recovery of added hydroxyl-amine
	Extn. by shaking	Extn. in cont. extr.	Extn. by shaking	Extn. in cont. extr.	
Phaseolus mungo (Green gram) ..	0·97	0·98	18·61	18·73	98
Vigna catjang (Karamani)	0·97	0·98	17·39	17·55	98
Phaseolus radiatus (Black gram) ..	0·98	0·99	16·49	16·67	99
Dolichos biflorus (Horse gram) ..	0·97	0·98	13·03	13·24	98
Pithecolobium saman (Rain tree) ..	0·98	0·99	21·21	21·44	99
Pongamia glabra (Pungu)	0·97	0·98	22·21	22·43	98

experiments in which 100 g. of root nodules of each species were used so that the estimations of free and combined hydroxylamine, the recovery of added hydroxylamine and the relative efficiencies of the methods of extraction could all be determined on the same sample.

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A NOTE ON THE BREEDING HABITS OF A COMMON EEL, *ANGUILLA BENGALENSIS* GRAY AND HARDW.

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(Communicated by Prof. A. Subba Rau, D.Sc.)

Hamid Khan from Punjab, Mukerjee from Calcutta, Sunder Raj from Madras have done some useful work on the development and breeding habits of some fresh-water fishes and on marine fishes some valuable work has been conducted by the Madras and Travancore Fisheries Departments and also in the University Zoological Laboratory, Madras. Besides these Jones and Job have done some work of this nature on Larvicidal fishes also.

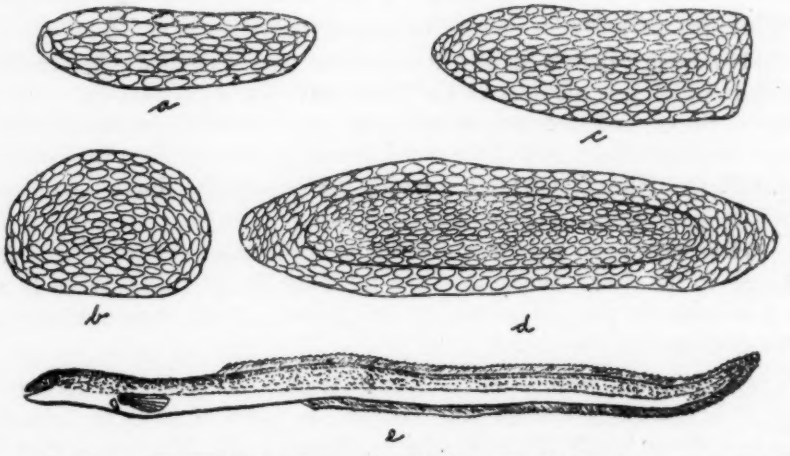
Regarding migratory fishes Hora has done extensive work on the anadromous fish, *Hilsa ilisha* Ham. which comes to fresh-water for breeding from the sea during the floods and he asserts that it breeds even in closed water, and does not migrate to the sea at all. On the other hand, Sunder Raj and Naidu who have been working on this fish in Cauvery, Godavari and Kistna are of opinion that it is essential for the *Hilsa* to go to the sea after breeding in fresh-water and its migration is essential.

In this paper we are dealing with a Catadromous fish *Anguilla* which belongs to the *Apodes* and Genus *Anguilla*. Extensive researches have been done on *Anguilla anguilla* and other allied species by many European workers and especially by the Danish Ichthologist Professor Schmidt who has traced the life-history in much detail. He was able to locate the breeding places near Sargasso sea in the Altantic very far from European coast. As it is well known, he has shown that *Anguilla* migrates from fresh-waters to the sea; gonads become mature only when it reaches salt-water; fertilisation takes place in the sea and *Leptocephalus* or larval stages migrate first with the currents towards the land, then finally enter fresh-water and change into the *Elver* stage which is a small form of *Anguilla*, the growth in size takes place in fresh-water. Further, the elver stage is reached after or about the age of 5 years and scale-formation starts only when they reach a size of about 8 inches.

In its geographical distribution eel shows very interesting features. The whole of America, South as well as the North, has no eels on its Pacific shores, whereas Japan, China, part of Australia and South East Africa have eels in most of their lagoons and rivers. In the fresh-waters which flow into

that sort of inland sea bounded on the north by Cochin China, on the west by the Malay Peninsula and the island of Sumatra, on the south by Borneo and Java, no sign of an eel is ever seen. On the other hand, there are swarms of eels in the rivers of Java and Borneo which empty themselves into the Indian Ocean.

It has been found that the regions which have eels in their fresh-waters are always bordered by seas of exceptional depth, or are visited by currents which pass over those depths. The situation becomes clearer when we remember that when eels are very young, while they are still fry, they come from the sea, that they begin life far out at sea and live there for a time before they begin to penetrate the rivers. They are originally deep sea creatures. Rising from the depths to approach the shore and enter the rivers, the particular part of the land to which they will go is determined by the part of the



a, *b* and *c*. ×50. Different patterns of scales from *Angulila bengalensis*, measuring 11". *d*. ×50. A scale from a specimen 19" in length showing one growth ring. *e*. ×1. Young specimen of the same fish measuring 6" having no scales.

sea from which they come. They can only approach the land when their home in the sea is within immediate reach.

The problem was suggested to us by Professor R. Gopala Aiyer as he was of opinion that belonging to the same genus and family our eels may also have the similar habits and will go to the sea for breeding.

During the month of September 1943 while on tour, one of us noticed swarms of small worm-like creatures which were trying to go up the current when the flood gates of Nizamsagar were opened. After catching some

they were found to be young ones of eels the smallest being 6 inches long. More specimens were caught and preserved. To ascertain their age, the skin was carefully scraped and it was found that the smallest specimens of 6" had no scale formation, but the scales were present on specimens of 11 inches and upwards. As will be clear from the three types of scales found on this specimen of 11", no annual rings have formed and if we accept the deductions in the case of European eels this most probably was about 6 years old as the scale formation starts only after 5 years.

Scales from a specimen of 19 inches were about double the size of the former and showed clearly only one growth ring which means that it may be a year older and of about 8 years of age. These are some of the results obtained after scale reading but further work regarding the growth rings on the otoliths is under progress. Collection of a large number of specimens is also contemplated when the condition of their gonads will be studied and further data will be collected.

Nizamsagar dam is about 450 miles from the sea situated across the River Manjra which is a tributary of the Godavari. The Godavari falls into the Bay of Bengal and there are anicuts near Rajahmundry, a precipitious ascent near Bhadrachellum and another anicut near Dummugudam; the course in H.E.H. the Nizam's territory is mostly rocky, and the flow of water very swift.

Now, arise some questions and doubts about the ascent of 'elvers' from the sea to the river. Where does the fish breed? If it does so in the sea, is it possible for the young ones of such a small size to travel a distance of 450 miles against the current?

Does the fish breed in fresh-water? We are of opinion that most probably it breeds in fresh-water and has left its habit of going to the sea as has been shown by Dr. Hora for the anadromous fish, *hilsa* that it does not go to the sea at all and it grows and breeds in the closed waters near Pulta Reservoir and other places.

It is an interesting problem and research workers situated near the estuaries will be in a better position to solve it as it would be a very remunerative concern for the fisheries if its breeding habits are fully worked out.

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A CONTRIBUTION TO THE LIFE-HISTORY OF *LITCHI CHINENSIS* SONN.

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Received May 13, 1943
(Communicated by Dr. S. N. Das Gupta, F.A.SC.)

Litchi chinensis, a member of the family Sapindaceæ is a native of China. It is said to have been introduced into this country and is at present widely cultivated as a fruit tree for its sweet arillous fruits of extraordinary flavour.

The literature on the morphology of plants belonging to the family Sapindaceæ appears to be meagre. According to Schnarf,[3] Guérin in 1901, published a paper on the development of the endosperm and embryo in *Cardiospermum Halicacabum*. Later Perrot and Guérin worked on *Alluandia* (*Didiera*).

In India Abdur Rahman Khan[2] worked on the pollination and fruit formation of Litchi. He has mainly described the different forms of flowers and has recorded his observations on the nature of the flowers produced in the different branches during the flowering season. Recently Sen[4] has made some morphological studies.

The interesting observations made by Khan[2] on the nature of flowering, and the dearth of any information regarding the embryology and cytology of the plant made it an important subject for study. A short note on some important observations was published in 1940 by one of us.[1]

Material and Methods

The material for this study was obtained from plants growing in private gardens at Ballygunge and also at the garden of the Agri-Horticultural Society at Alipur. The material was fixed in the garden, and an exhaust pump was always used to ensure proper penetration of the fixing fluid. Allen's modified Bouin's fluid and Flemming's fluids were used for fixation. Dehydration, clearing and embedding were done in the customary way. Sections were cut 10 to 16 microns thick depending on the stage required for study. Heidenhain's iron-alum hæmatoxylin and Newton's gentian violet iodine were used for staining.

(*a*) *The flower and the inflorescence.*—The flowers are functionally unisexual. The floral morphology of the different types of flowers is described below :—

(i) *Female flowers.*—Calyx usually 4-lobed, dentate. Corolla absent. Disc fleshy, glabrous. Stamens 6, filaments short, pubescent, inserted inside the disc and around the pistil. Anthers turgid, non-dehiscent. Gynæcium pubescent. Ovary 2-lobed, style prominent. Stigma bifid with well-developed glandular hairs.

(ii) *Male flowers.*—Calyx usually 4-lobed. Corolla absent. Disc glabrous, smaller than that in the female flower. Stamens usually 6, rarely 7 or 8. Filaments long, pubescent. Gynæcium in the form of a pistillode which is thickly covered with hairs.

Another type of male flower which varies slightly from the aforesaid type has been observed in some trees. In this, the filaments of the anthers are shorter than in normal male flowers. It has a pistillode which outwardly resembles a fertile gynæcium. Rudimentary ovules are present inside the pistillode. The style is short and the stigmatic lobes remain unopened.

All the flowers are pedicillate. The pedicel and the calyx lobes are hairy. In some of the female flowers the filaments become very short and bear two anthers. Of these, one anther lobe of each anther is bigger than the other. In other instances the filaments become branched and each of them bears an anther.

The inflorescence is a panicle. The flowers are borne on the axis of the 3rd order, rarely on the 4th, and occasionally on the main axis of the inflorescence. Generally speaking, the inflorescence is of a compound racemose type. The flowers occur in cymes, which are generally of one sex. The constitution of the cymes with regard to the sexuality of the flowers often varies during the flowering season. Generally variations are noted during the transitional stages of flowering, *i.e.*, when both male and female flowers occur together. The different types of cymes observed are as follows:

(i) Cymes composed entirely of male flowers.

(ii) Cymes composed entirely of female flowers.

(iii) Cymes terminated by a male flower with the lateral flowers female.

(iv) Cymes terminated by a female flower with the lateral flowers male.

(v) Cymes terminated by a male flower with the lateral flowers of different sexes.

(vi) Cymes terminated by a female flower with the lateral flowers of different sexes.

All the branches of the inflorescence (with rare exceptions) begin flowering almost simultaneously. The flowering as noted by Khan[2] is characterised by the opening of flowers of different sexes at different periods. Flowering records of a few plants for two consecutive seasons (1940 and 1941) show that there are distinct periods of bloom for the male and the female flowers. These periods may occur alternately or two consecutive periods may overlap.

The majority of the trees produce male flowers first. The number of 'flushes' in the different trees appears to be variable and in some instances a transitional stage is also observed.

Similar observations have been made by Khan.[2] It is interesting to note that the flowering period is very short and is composed of succession of flushes of male and female flowers. Table I shows the duration of flowering of the trees under observation during the seasons 1940 and 1941.

TABLE I

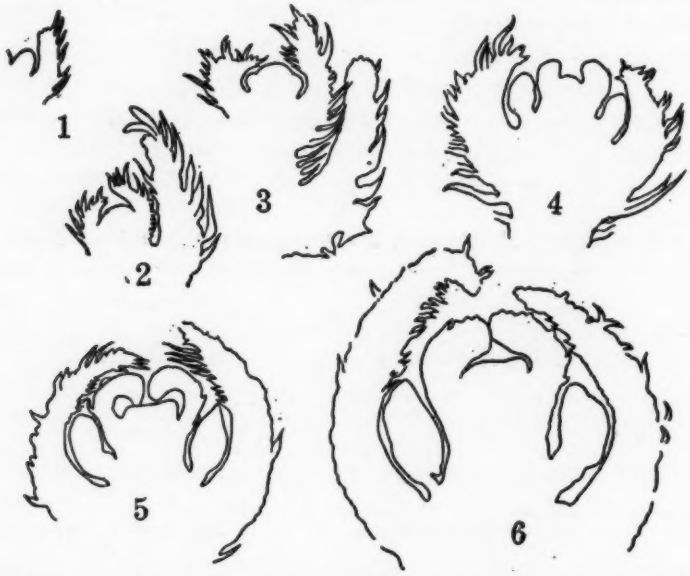
Litchi chinensis. Duration of Flowering in the Individual Trees

Tree No.	1940	1941
1	12th Feb. to 9th March (27 days)	Did not flower
2	15th Feb. to 10th March (25 days)	25th Feb. to 19th March (23 days)
3	20th Feb. to 4th March (14 days)	23rd Feb. to 11th March (17 days)
4	3rd March to 22nd March (20 days)	10th March to 22nd March (13 days)

Thus the average period of flowering in the year 1940 was 21·5 days and 14·3 days in 1941. Khan[2] found the average period to be 24·25 days in the year 1928. It must be admitted that these observations are of a preliminary nature and it is desirable that observations of a large number of plants extended over a few years should be made to get a clear idea as to the period of flowering and also on the influence of climatic factors on the production of flowers.

(*b*) *The organogeny of the flower.*—The flower primordium first makes its appearance in the axil of a bract (Text-Fig. 1). The primordium soon

enlarges and from its sides protuberances arise, which grow rapidly and develop into the calyx lobes (Text-Fig. 2). These are four in number as seen in transverse sections. The calyx lobes are characteristically hairy, the hairs are unicellular and are the prolongations of the epidermal cells. They are thick-walled, curved and subulate in form. At this stage the central primordium appears as a semicircular mass of tissue in longitudinal sections, so that it is really convex in form. The primordia of the anthers next become differentiated at the axil of the calyx lobes and away from the centre (Text-Fig. 3). The central primordial tissue grows slightly, but very soon two lateral protuberances develop and the tip becomes somewhat flattened (Text-Fig. 4). These protuberances are the carpel



TEXT-FIGS. 1–6 (×50). *Litchi chinensis*.—Fig. 1. Appearance of the flower primordium. Fig. 2. The origin of the calyx. Fig. 3. The promordia of the stamens appear. Fig. 4. The growth of the stamens and appearance of the carpel primordia. Fig. 5. Further growth of the carpel primordia. Fig. 6. A later stage of the same, showing the presence of a gynophore.

initials which curve over and meet at the central region of the floral axis where the tissues subsequently become united. The later growth of the carpels takes place both in dorsal and ventral directions (Text-Fig. 5). Thus the solid style and the bilocular ovary is organised. The tissues encased by encircling carpels form the placenta. The stigma is bifid, the lobes are recurved and show the presence of glandular cells.

Simultaneously with the development of the carpels the tissue lying between the stamens and the ovary grows in length resulting in the formation of a short gynophore (Text-Fig. 6). A disc is also noted to develop between the bases of the growing filaments and calyx lobes. It assumes a massive form when fully developed.

(*c*) *The development of the microspores and the tapetum.*—The microspore mother cells soon after their differentiation are closely packed inside the anther loculus. At synizesis a contracted knot is produced which generally encloses the nucleolus in its meshes. The bivalent nature of the chromosomes becomes apparent at diakinesis (Text-Fig. 7). Division I is regular, though occasionally a few laggards have been noted. During interkinesis the chromosomes show the split for the II division. Cytokinesis takes place by the process of furrowing. No difference in the meiotic process was noted in the pollen mother cells of the functional female plants.

The differentiation of the tapetal layer in the microsporangium could not be traced. The tapetum is first recognised when the microspore mother cells have become organised. It consists of a single layer of cells, each cell containing a big nucleus and granulated cytoplasm (Text-Fig. 9). Along with the growth of the microsporangium the tapetal cells divide mitotically without the formation of a wall, as a result, the cells become binucleate. At this stage the cells increase in a radial direction and seem to protrude inside the anther cavity (Text-Fig. 10). This condition is generally maintained till the formation of the microspores when the tapetal cells grow laterally and become more or less rectangular in form as shown in Fig. 11. The tapetal cells degenerate after the pollen grains are fully differentiated.

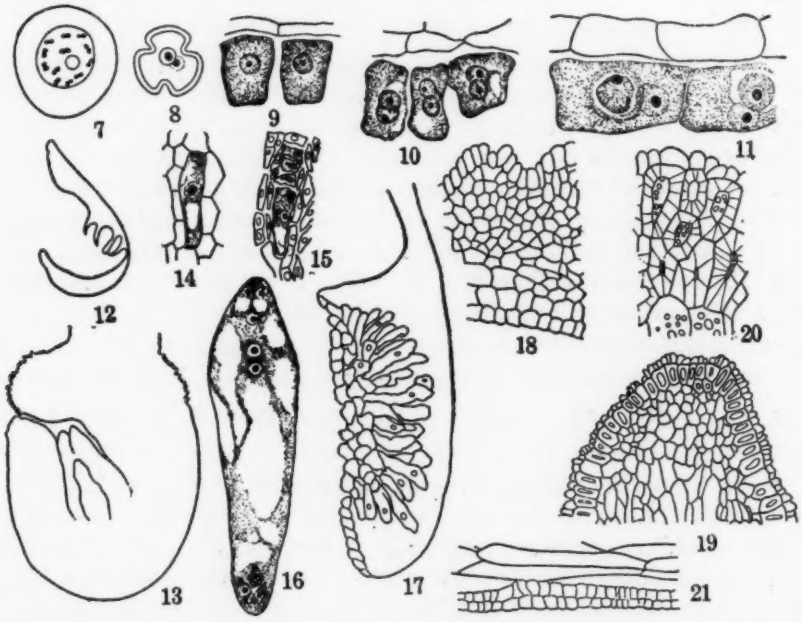
(*d*) *The pollen grains.*—Pollen grains obtained from the male flowers are barrel shaped when dry. Mounted in water or lactic acid, they become somewhat triangular in form, showing the presence of three germ pores at the corners. The exine shows the presence of three furrows in the centre of each of which a germ-pore appears to be present. The exine is granular at the surface and its colour light yellow. Comparative measurements of pollen grains obtained from each type of flower shows very little difference in size.

The pollen grains of the male flowers are binucleate at the time they are shed. The two nuclei lie very close together and appear to be almost identical in shape and size (Text-Fig. 8).

Attempts were made to germinate the pollen grains under laboratory conditions. Five per cent. cane sugar solution was used as a medium and

the cultures were kept under humid conditions. The pollen grains germinated within an hour. The male flowers produced normal pollen tubes, whereas the female flowers produced "short tubes".

(*e*) *The development of the female gametophyte.*—The ovule primordium first arises as a tiny protuberance from the placental tissue. It grows obliquely upwards and very soon assumes an anatropous form. The ovules are bitegmic. The outer integument is absent at the ventral side of the ovule where it is congenitally fused with the funicle. At this region a 'hump-like' obturator is present (Text Fig. 12). It becomes differentiated simultaneously with the integuments but appears to grow faster. In the mature ovule the obturator lies just above the inner integument and close to the outer as represented in Text-Fig. 13.



TEXT-FIGS. 7–21. *Litchi chinensis.*—Fig. 7. Diakinesis (P.M.C.). × 775. Fig. 8. A binucleate pollen grain. × 775. Fig. 9. Uninucleate tapetal cells. × 775. Figs. 10 and 11. Later stages of tapetal cells. × 775. Fig. 12. An early stage of the development of the ovule and obturator. × 60. Fig. 13. A later stage of the same, showing the origin of aril. × 60. Fig. 14. The megaspore mother cell. × 1250. Fig. 15. A linear tetrad of the megaspores × 750. Fig. 16. A mature E.S. × 490. Fig. 17. Initiation of aril development. × 225. Figs. 18–21. Illustrate the tissues found in the different regions of the fruit. Fig. 18. × 225. Fig. 19. × 75. Figs. 20 and 21. × 225.

The origin of the archesporial cells could not be definitely traced. The megaspore mother cell when first observed is comparatively large and occurs deep inside the nucellus (Text-Fig. 14). It passes through the usual stages of reduction division and produces a tetrad of megaspores (Text-Fig. 15) of which the chalazal one becomes functional and the others degenerate. Sometimes the three non-functioning megaspores persist upto the one-nucleate stage of the embryo-sac. The functional megaspore increases in size and by three successive divisions produce an eight-nucleate embryo-sac.

The mature embryo-sac is of the normal angiospermous type (Text-Fig. 16). The synergids are pear-shaped structures and show the presence of a vacoule at the lower end. The egg protrudes beyond the synergids. The secondary nucleus lies close to the egg and is comparatively big. The antipodals are ephimeral.

The endosperm is of the "nuclear type".

(*f*) *The development of the aril and the fruit.*—In the mature ovule an obturator is present at the micropylar region. From this commences the development of a tissue which later forms the aril (Text-Fig. 17). In lorgitudinal sections of the fruit a similar outgrowth is seen on the other side of the funicle (Text-Fig. 13). Thus it appears that the primordium of the aril extends all around the seed. The growth of this tissue is not uniform and as a consequence portions of the aril are seen to overlap at the chalazal end of the seed. At first the tissue composing the aril consists of closely packed parenchymatous cells. Soon the cells increase in size, become elongated and show the presence of intercellular spaces. It should be noted that the development of the aril and the fruit do not proceed simultaneously in every instance as in many well-developed fruits the aril was noted to be in a rudimentary condition.

As has been noted by Khan[3] fruits are generally developed from one loculus of the ovary. In rare instances, however, both the loculi develop into fruits. The orientation of the fertile loculus during the development of the fruit is a noteworthy feature. The fertile loculus, during its development, bends outwards and towards the axis of the flower and ultimately comes to lie in the same plane as the latter. The sterile loculus and the style being deflected from their original position remain at the base of the fruit.

The mature fruit of Litchi is crimson coloured. It is indehiscent nut-like and tuberculed. Anatomical studies of the developing fruits show that the tubercules develop from the wall of the ovary from which the hairs had fallen off after fertilisation. Changes in the outer wall of the ovary are noted

during the later stages of the development of the female gametophyte. The epidermis becomes wavy in appearance due to localised division of certain cells in the hypodermal region. The epidermal cells also divide to accommodate the tissues below (Text-Fig. 18). In this way the tubercules are produced. Along with the growth of the tubercules and the development of the fruit, the cells composing it also become differentiated. The tissues found in the different parts of the pericarps of the fruit are as follows:—

(1) *Epicarp.*—The tubercules contain the tissues of the epicarp. Four distinct types of tissues are noted: (i) The epidermis composed of rectangular cells with slightly thickened outer walls. (ii) Hypodermal sclerotic cells, with characteristic transverse striations. These cells occur in one, two or three layers and show the presence of tanin inside: (iii) Ordinary parechymatous cells with slightly lignified walls and dense cell contents similar to the above. (iv) Thin-walled parenchymatous cells occur at the base of the tubercle. Special cells containing "resinous or latex-secretions" occur mostly at the sides (Text-Figs. 19 and 20).

(2) *Mesocarp.*—The mesocarp tissue is somewhat loose in consistency. There does not appear to be any line of demarcation between the epicarp and the mesocarp. In the initial stages this tissue is composed of ordinary paranchymatous cells which later becomes separated on account of the development of large intercellular spaces.

(3) *Endocarp.*—Two distinct tissues have been noted. (i) The outermost one (*i.e.*, next to the aril) is composed of two layers of rectangular cells which are considerably thickened and lignified. (ii) The tissue above it is composed of thick-walled elongated prosenchymatous cells with tapering walls (Text-Fig. 21).

The vascular supply of the fruit is confined to the tissues of the mesocarp. If the tubercules of a ripe fruit are carefully removed a white membranous structure is seen enveloping the aril. The impressions of vascular strands are noted on this structure which seems to be composed of the tissues of the endocarp. Sometimes the tissues of the mesocarp with adhering vascular bundles are seen attached to this tissue.

Summary

The paper gives an account of some aspects of the life-history of *Litchi chinensis.*

1. The flowers though apparently bisexual behave functionally as male and female. In the female flowers the filaments are short and the

anthers do not burst. In the male flowers on the other hand, the pistil does not attain full development and remains as a pistillode.

2. The inflorescence is a panicle. It bears male and female flowers alternately. Normally female flowers appear after the male flowers without any break. The limits of the flowering periods may or may not overlap. The flowering period as a whole is very short.

3. The development of the floral parts occurs in the following sequence calyx, andrœcium and gynæcium. The development of the gynophore is first noted during the differentiation of the gynæcium.

4. Development of the pollen grains is of the simultaneous type. Meiosis in the pollen mother cells of the functionally male and female flowers does not show any difference or irregularity. Cytokinesis takes place by the process of furrowing. The tapetal cells are binucleate.

5. The pollen grains of both the types of flowers appear to be similar in shape and size. The exine is granular and shows the presence of three furrows, in the centre of each of which a germ-pore appears to be present. The pollen grains are binucleate at the time they are shed.

6. The development of the female gametophyte is of the normal type. The mature embryo-sac is eight-nucleate. The antipodals are ephimeral.

7. The aril develops from the funicle and the tissue of the obturator takes part in the process.

8. The pericarp consists of an epicarp, mesocarp and endocarp. The cells of the epicarp develop into tubercules and show tissue differentiation. The mesocarp appears to be continuous with the epicarp. The endocarp is composed of two different types of tissues.

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THE EFFECT OF CERTAIN EXTERNAL FACTORS UPON THE GROWTH OF WHEAT

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I. Introduction

The importance of growth and development studies in crop plants as has been pointed out by Engledow (1933) hardly needs any emphasis. This is one of those agronomic problems upon the solution of which depends the progress of Agriculture. A variety may not be able to express its genetical potentialities to the fullest extent under one set of conditions. In the present investigation an attempt has been made to study the influence of certain external factors upon the growth of wheat in the hope that the results may throw light on this important problem.

A review of the considerable literature on developmental studies in wheat shows that comparatively few investigators have studied the wheat plant from this angle. Mention must, however, be made of Grantham (1917) who made a study of environmental factors such as time, rate and depth of seeding, on tillering. He concluded that environments had a great modifying influence on the wheat plant.

II. Materials and Methods

Three varieties of *Triticum vulgare* Host, *viz.*, Punjab 8 A, Pusa 52 and Pusa 165 were used in the present investigation. Studies were made on Punjab 8 A during the year 1933–35 at Lyallpur and on the two Pusa varieties during 1935–37 at Pusa. Punjab 8 A and Pusa 52 are bearded and prostrate in habit while Pusa 165 is beardless and erect. The experiments which were carried out are described below:—

(i) Experiment to investigate the influence of soil, season and depth of sowing on the germination of wheat seeds

Place	Year	Material	Lay-out
Lyallpur ..	1933–34	Punjab 8 A wheat Three soils, namely, light loam, loam and heavy loam	Randomised blocks with six replications; 100 seeds per plot sown in three different soils at 10 depths: ½, 1, 1½, 2, 2½, 3, 3½, 4, 4½ and 5 inches.
Lyallpur	1934–35	(i) do. (ii) Punjab 8 A ; loam	(*a*) do. (*b*) As (*a*) above but sown on 21 different dates from September to January. (*c*) As (*a*) above but sown on 15–10–34 and 15–11–34 at 8 P.M., 2 P.M. and 5 P.M.
Pusa ..	1936-37	Pusa 52 and Pusa 165 ; light loam	(*a*) Randomised blocks with six replications ; 30 seeds per plot sown on 30–10–36 at depths of 1, 3 and 5 inches. (*b*) Randomised blocks with six replications ; 20 seeds per plot sown on 5–11–36 at depths of 1, 2, 3, 4, 5 and 6 inches. (*c*) As (*b*) above but sown on 10–11–36 and 11–11–36 at depths of 1″ and 2″ at 7 P.M., 12 noon and 5 P.M. (*d*) As (*a*) above but sown on 12–12–36.

(ii) Experiment to investigate the relation of size of grain and germination when sown at different depths

Place	Year	Material	Lay-out
Pusa ..	1936–37	Pusa 52 Pusa 165	Randomised blocks with six replications ; 50 seeds per plot, sown on 10–12–36 at depths of 1, 2, 3, 4 and 5 inches.

(iii) Experiments to investigate the influence of sowing depth, space and season on growth of wheat

Place	Year	Material	Lay-out
Lyallpur ..	1933-34	Punjab 8 A wheat	(*a*) Randomised blocks with three replications ; 80 seeds per plot sown on 16 different dates at weekly intervals beginning from 8–10–33 at depths of 1, 2, 3, 4 and 5 inches. The spacing from plant to plant was 12″ × 12″.

(iii) Experiments to investigate the influence of showing depth, space and season on growth of wheat—(Contd.)

Place	Year	Material	Lay-out
Lyallpur ..	1934-35	Punjab 8 A wheat	(*b*) Randomised blocks with three replications ; 60 seeds per plot sown on 20 different dates at weekly intervals beginning from 17-9-34 at depths of 1, 2, 3, 4 and 5 inches, and at plant to plant spacing of 3, 6, 9, 12 and 15 inches. (*c*) Randomised blocks with six replications ; 80 seeds per plot sown on 9 different dates at fortnightly intervals beginning from 17-9-34 at depths of 1, 2½ and 5 inches, and at plant to plant spacing of 3, 9 and 15 inches in an artificially prepared plot. The spacing from row to row in (*b*) and (*c*) was 9 inches. (*d*) Randomised blocks with six replications ; 6 fortnightly sowings beginning from 15-10-34 with local plough at depths of 1½, 2½ and 5 inches at plant to plant spacing of 3, 9 and 15 inches. (*e*) Randomised blocks with six replications ; sowing with local plough on 17-11-34 at depths of 1½, 2½ and 5 inches ; normal seed rate used. (*f*) Randomised blocks with six replications ; sowing with local plough on 17-11-34 at plant to plant spacing of 3, 9 and 15 inches at normal depths. (*g*) Randomised blocks with six replications ; sowing on 17-11-34 with local plough at normal depths ; seed rate : 8, 16, 24 and 32 seers per acre. Area of each sub-plot in case of (*d*) was 1/200 of an acre and in (*e*)to(*g*) it was 1/54th of an acre.
Pusa ..	1935-36	Pusa 52 Pusa 165	(*a*) Randomised blocks with six replications; 50 seeds per plot sown on 29-10-34.
do.	1936-37	do.	(*a*) Do. as above except 100 seeds per plot sown on 29-10-36, 12-11-36, 26-11-36 and 10-12-36.
	do.	do.	(*b*) Randomised blocks with six replications ; 20 to 100 seeds per plot sown on 30-10-36 at plant to plant spacing of 3, 9 and 15 inches. (*c*) As (*b*) above but sown on 12-12-36.

III. Experiments and Results

Different plant characters may differently respond to given environmental factors. In the present investigation the following plant characters were studied under different dates and time of sowing, different depths of sowing, different conditions of spacing, and different kinds of soil:

(1) Germination, (2) survival of seedlings, (3) formation of first four foliage leaves and side-tiller, (4) number of leaves per tiller, (5) number of tillers per plant, (6) plant height, (7) emergence and ripening of ear, (8) length of ear, (9) number of fertile and sterile spikelets, (10) number of grains per ear, (11) size of grain, (12) weight of grain, (13) quality of grain and straw, (14) grain flinty or mealy and (15) yield.

The results obtained in respect of these characters are described in this paper, each of the fifteen characters mentioned being considered seriatim.

1. *Germination*

(*i*) *Effect of kind of soil and depth of sowing on total germination.*—Seeds were sown at ten depths in three different soils. Statistical analysis of the data showed that sowing depth had exercised the greatest effect in the experiment. The interaction between soils and depths was also significant. The final germination percentages obtained for Punjab 8 A wheat in all the three soils are given below:—

Soil	Sowing depths									
	½″	1″	1½″	2″	2½″	3″	3½″	4″	4½″	5″
Heavy loam	62	96	100	94	92	84	72	56	50	44
Loam	60	95	94	98	96	86	76	62	54	50
Light loam	52	94	96	98	98	96	86	78	70	66

The underlined figures indicate the ranges of optimum depths under each condition. The optimum depths for heavy loam, loam and light loam soils are about 1½″, 2″ and 2½″ respectively. At Pusa where the varieties Pusa 52 and Pusa 165 were sown at different depths the results were in conformity with those obtained at Lyallpur except that at greater depths greater germination percentage was obtained than at Lyallpur, *e.g.*, at 5″ depth, 78 per cent. germination was obtained against 66 per cent. at Lyallpur.

(ii) *Effect of season and depth of sowing on total germination.*—Twenty-one sowings of Punjab 8 A wheat were made at Lyallpur in loam soil from September 1934 to January 1935. The object of this experiment was to study the effect of season on the time of start and completion of germination as well as its percentage (germination) when seeds were sown at different

depths. Statistical analysis of the data showed that except for blocks the effect of all other variables was significant. When the data of the different dates were compared it was found that the optimum ranges were the same in most cases. The datailed data of only those dates are set forth below the ranges of which were different from one another:—

Sowing dates	Sowing depths										Mean daily temperature	Relative humidity percentage
	½″	1″	1½″	2″	2½″	3″	3½″	4″	4½″	5″		
17 -9-34 to 9-10-34 ..	52	84	96	98	94	90	82	72	64	60	81·2	58·5
16-10-34 to 15-11-34 ..	56	90	94	98	98	96	82	68	62	56	65·1	83·1
18-11-34 to 3-12-34 ..	72	100	98	100	96	94	88	64	56	50	61·0	89·1
8-12-34 to 14- 1-35 ..	84	91	92	92	88	86	72	58	52	41	45·3	82·3

Though the optimum range of 18/11 to 3/12 is not appreciably different from that of 16/10 to 15/11, but it shows that during this period germination percentage increases at shallow depths and hence sowings should preferably be made at shallower depths.

It is obvious that the optimum range of sowing depths varies with the date of sowing.

(iii) *Effect of season and depth of sowing on time of start and completion of seed germination.*—The time of germination was obviously affected by sowing at different dates and depths. The seeds at shallower depths and in the earlier sowings germinated earlier than those at greater depths and sown later in the season. These differences were intensified with increase in depth and with the delay in time of sowing. The figures for sowings made on five different dates are given below:—

Average number of days required for the time of start (S) and completion (C) of seed germination of Punjab 8 A *wheat in sowings made at different depths and dates*

Sowing dates	Sowing depths										Mean daily temperature	Relative humidity percentage
	1″		2″		3″		4″		5″			
	S	C	S	C	S	C	S	C	S	C		
22- 9-34 ..	4	6	4	6½	4	7	4½	7½	5½	8½	87·1	67·8
5-11-34 ..	5½	8½	6½	8½	6	8½	6½	9	7	11	67·5	79·0
3-12-34 ..	8	12	8½	13	8½	13	9	13½	9½	15½	61·0	89·1
27-12-34 ..	11½	15	12	17	12½	18	13	19	14	20½	57·3	89·7
14- 1-35 ..	15½	21½	16	24½	16½	26	17½	28½	18½	29	45·3	82·3

These data lead to the important practical conclusion namely that to compensate for delay in time of sowing, seeds should be sown at shallower depths. Thus same conclusions were arrived at from the investigations carried out at Pusa but for the sake of simplicity of presentation the detailed data are not separately presented here.

(iv) *Effect of time of sowing on total germination and on rate of germination.*—Seeds were sown on various dates from September to January in three different soils and at 3 to 10 different depths at three times of the day, namely morning, noon and evening. It was observed that time of sowing had practically no effect on total germination. But it was also noticed that under whatever conditions sowings at Pusa and Lyallpur were made the seeds always began to germinate at about the same time, *i.e.*, in the cool hours. This is an interesting finding and the explanation appears to be that seed remains in soil for a certain period before germination can take place and if this period ends when the hours to follow are favourable for germination it begins to germinate failing which it waits for favourable conditions to come.

During noon hours rate of germination was very slow and the least germination took place in the afternoon upto about 8 P.M. The greatest number of seeds germinated during the cool hours, *i.e.*, after midnight irrespective of the time and conditions under which the sowings were made.

(v) *Relation of size of grain to germination.*—While studying the rate of germination an interesting point was noticed namely that Pusa 165 which has bolder seeds than Pusa 52 germinated at a slower rate at shallower depth as compared with Pusa 52. At greater depths however the positions were reversed. Pusa 165 also gave higher germination percentages at the greater depths than Pusa 52. An experiment was therefore laid out to study if this difference in behaviour could be ascribed to the size of seeds. The difference was quite discernible within a variety at all the depths, the greater germination percentage being in favour of bolder seeds. But the higher rate of germination of Pusa 52 at shallower depths seems to be a varietal characteristic.

2. *Survival of Seedlings*

(i) *Effect of sowing at different depths and spacings on survival of seedilngs.*—There is a common belief in the Punjab that sowings early in the season generally suffer due to the high day temperature prevailing at that time, resulting in a high mortality of seedlings. These investigations carried out both at Lyallpur and Pusa have definitely shown that spacing from plant to plant has practically no effect on mortality. The percentage

survival had been low at the shallow depths and especially at the 1 inch depth but from the third week of October survival percentage goes on increasing till by the middle of November there remains little difference between the various depths.

(ii) *Effect of sowing on different dates on survival of seedlings.*—Survival percentage of wheat seedlings at different times after sowing when sowings were made at Lyallpur during the year 1934 is shown in Fig. 1.

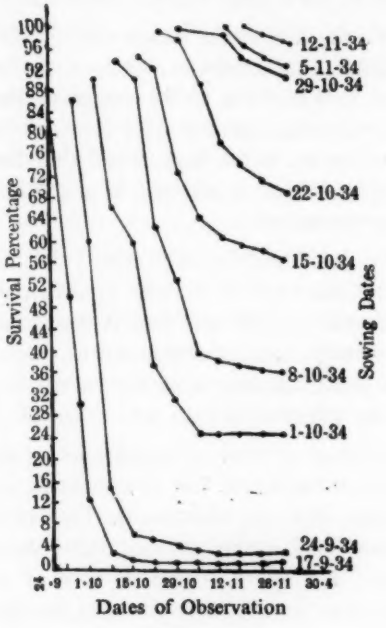

FIG. 1

Survival percentage of wheat seedlings at weekly intervals

Curves have not been made for the data of sowings made after 12th November because there was practically no mortality in those sowings. It is obvious that mortality is very high in all the sowings made upto 8th October, but a definite improvement occurs in the next sowing. The practical application of these observations is that to obtain a good stand of the crop it is necessary to vary the seed rates according to the time of sowing.

Death of seedlings takes place sooner after sowing in early sowings as compared with late sowings. For instance most of the seedlings died out within a fortnight after sowing in the case of the earliest sowing. It has

been observed that once the seedlings get to be five weeks old, they generally survive and live to yield a crop.

Among the probable causes of such a high mortality in early sowings are the attacks of white ants and *Helminthosporium sativum* (Mitra and Bose, 1935). The optimum temperature for maximum development of the latter is 25-30° and this is usually attained in the months of September and October.

3. *Rate of Formation of First Four Foliage Leaves and Side-Tiller*

The rate at which the first green leaves and the first side-tiller become visible is a well-defined characteristic. Counts made hourly, daily and weekly at Lyallpur as well as Pusa in the course of these studies indicated that there is a high correlation between the time taken for germination of seeds and formation of leaves. It has been found that the rate of appearance of the first-formed foliage leaves is affected in a similar way by the factors influencing rate of germination.

First foliage leaf.—As already stated (*supra*) the rate at which the first leaf appeared was about the same as the rate at which the seeds germinated. But an interesting observation made was that it was generally longer, thicker and broader in early sowings, and at depths up to three inches as compared with late sowings and greater depths, with the exception that in late sowing, *i.e.*, after November the effect of depths was reversed.

Second leaf.—The range of time of appearance of the second leaf under all the conditions was comparatively less as compared with third and fourth leaves. Two to four days after germination the formation of the second leaf started and it was completed within two or three days. Spacing had no effect on its formation. The effect of season, time of sowing and depth of sowing was almost the same as that described for the first leaf (*supra*).

Though Pusa 52 germinates earlier than Pusa 165, leaf formation commenced earlier in the latter. This also seems to be varietal characteristic and the association between time of germination and appearance of leaves, etc., as stated above holds good only within a variety.

Third leaf.—Its range of time of appearance was much longer than that for the first and second leaves. It began to appear 6–14 days after germination and was completed within 4 to 10 days. Its range, etc., increased with delay in sowing and with greater depths. Spacing did not affect its range or time of appearance.

Fourth leaf.—The range of time of appearance of fourth leaf was even wider than that for the third leaf. Its formation started from ten to fifteen

days after germination and was completed within six to ten days. In both early and late sowings at sowing depths of 1–3 inches, it was formed with or before the first side-tiller. But in late sowings at 5 inches depth it was always formed after the side shoot had appeared. In early sowings this result of the depth of sowing was not observed. The effect of other external factors was similar to that on the first three leaves.

Late sowings made at shallower depths tended to produce leaves quicker than those done at deeper sowings, the difference being 3-4 days in favour of shallower sowings (1" and 2"). This difference was not so noticeable in early sowings.

First side-tiller.—The range of time of appearance of the first side-tiller was not much greater than that for the fourth leaf. However, it went on increasing as the sowings were delayed. Its range of time of appearance varied from after formation of the 3rd leaf to before that of the 5th leaf. No case was encountered where it was formed before the 3rd leaf although a few cases were noted where it was formed with or just after the 5th leaf. The number of days after germination after which the 1st side-tillers were formed both at Pusa and Lyallpur are given below:—

Sowing dates

	Sowing dates (19 –)									
	17/9	1/10	15/10	29/10	12/11	26/11	10/12	24/12	7/1	21/1
Lyallpur—										
Punjab 8 A	30	26	21	19	27	28	31	35	42	45
Pusa—										
Pusa 165	..	..	..	18	20	25	25	..	..	..

The effect of other external factors both at Lyallpur and Pusa was similar to that on the appearance of fourth leaf.

4. *Number of Leaves per Tiller*

Pusa 165 has an erect early habit whereas Pusa 52 and Punjab 8 A have a procumbent early habit. In erect varieties the tillers are taller and the number of leaves per tiller is, therefore, more than in case of prostrate varieties in the early stages of growth. The number of leaves per plant in early stages does not directly correspond to the number of tillers per plant when erect and prostrate varieties are compared.

(i) *Effect of sowing at different dates.*—Late sowings tended to decrease the number of leaves produced per tiller though to a very slight extent. In

September—November sowings the number of leaves per tiller was 8-7 while in December and January sowings it was 7-5. (In January it was the least.)

(ii) *Effect of other external factors.*—Locality, season and spacing did not cause any appreciable difference in the number of leaves per tiller.

It is obvious that the effect of the favourable factors for vegetative growth, such as a long growing season, wide spacing of individual plants, etc., is expressed in vigorousness of growth of stem, leaves, number of tillers and not in the number of leaves per tiller.

5. *Number of Tillers per Plant*

Tillering, taken to mean the number of side-shoots (including the main axis of the plant) was recorded weekly up to harvest both at Lyallpur and Pusa. As tillering is a good criterion for the judgment of response to external factors in wheat, the data in respect of this character are given in some detail in the following pages.

A. *Total Number of Tillers.*—Statistical analysis of the data showed that the mean squares contributed by sowing dates were the largest and this variable produced the greatest effect in all the experiments. The effect of spacings, depths and differential response between dates and depths, and between spaces and dates were also significant. The effect of all other variables was insignificant.

Data collected both at Lyallpur and Pusa are given in Table I.

TABLE I

Comparative number of tillers per plant of the Punjab 8 A *wheat grown at Lyallpur and Pusa during* 1933–35 *at different depths and spaces*

Spacing from plant to plant	Sowing depths	Sowing dates (1933–34)							
		15/10	29/10	12/11	26/11	10/12	24/12	7/1	21/1
12″×12″	1″	104·0	78·0	53·3	42·0	24·7	10·0	4·0	3·3
	3″	101·0	76·1	52·0	38·0	19·0	7·5	2·9	2·3
	5″	96·2	71·1	40·3	30·0	14·3	6·0	2·0	1·8
		1934–35							
9″×3″	1″	25·3	21·7	15·4	12·4	8·6	4·9	3·2	2·9
	3″	26·4	24·5	17·8	13·7	7·1	3·1	2·4	1·8
	5″	22·2	18·1	11·1	7·2	3·2	1·5	1·1	1·1
3″	2″	25·8	23·2	17·4	13·1	7·1	4·1	2·9	2·3
9″		44·4	33·4	27·2	21·2	12·2	5·7	3·4	2·7
15″		54·2	74·2	48·2	31·9	15·4	8·0	4·1	3·0

Pusa (1935–37)

Variety	1935–36 29/10	1936–37 29/10	12/11	26/11	10/12	
Pusa 52 ..	13·2	13·9	12·7	14·4	10·3	Spacing from plant to plant 12″×6″. Sowing depth normal.
Pusa 165 ..	12·3	10·6	13·2	13·7	10·2	

(i) *Effect of depths.*—Examination of the results suggested that sowing depths had practically no effect on tillering in early sowings. But in December and January sowings the effect of greater depths became very much more marked and during these months seeds should be sown at shallower depths.

The regression of number of tillers on sowing depths was estimated. The regression co-efficients were significant only in late sowings done after the middle of November. Effect of spacings in the case of different depths seemed to be almost of the same trend. Of course, the number of tillers being greater at wider spaces the co-efficient values were also greater at the wide spacings.

(ii) *Effect of spacing.*—The number of tillers per plant at wider spacings is more than at narrower ones. But it is worth noting that if the number of tillers per unit area is compared the closest spacing appears to be the most effective for tiller production. This point will be clear from the following statement:—

	Spacing from plant to plant (1934–35)				
	3″	6″	9″	12″	15″
No. of tillers per plant observed in sowing made on 15–10–34 ..	25·6	37·4	44·4	49·1	54·2
No. of tillers calculated in sowing made on 15–10–34	25·8	51·6	77·4	103·2	129·0
Percentage increase	..	38·0	75·0	110·2	138·1
No. of tillers per plant observed in sowing made on 7–1–35	2·9	3·0	3·4	3·8	4·1
No. of tillers calculated in sowing made on 17–1–35	2·9	5·8	8·7	11·6	14·5
Percentage increase	..	93·3	156·0	205·3	253·7

It is evident that wheat sowings should always be made thick. Further it is brought out that the percentage difference between every two respective

spacings, *e.g.*, 3″ and 6″, 6″ and 9″, etc., etc., goes on increasing as the sowings are delayed. It means that wider spacings do not show corresponding increase in number of tillers in late sowings.

The regression of number of tillers on spacings was estimated. It was seen that rate of increase went on decreasing with delay in sowing dates. The values of the regression co-efficients for January sowings were almost insignificant. The differences in the regression co-efficients for different depths were insignificant in most of the cases, and did not lead to any definite and tangible conclusion.

(iii) *Effect of sowing at different dates.*—The investigations made at Lyallpur showed that there is a very high negative correlation between dates of sowing and number of tillers per plant. In October sowings the greatest number of tillers per plant was obtained; in January sowings it was the least. Regression co-efficients, *i.e.*, the rate of decrease of tillers for delay in sowing, was almost the same at all the depths in the same year but in spacing its value increased with increase in spacing. It was seen that on the whole as the spacing increased the rate of decrease of tillering per week's delay in sowing increased.

At Pusa except that date of sowing did not cause a noticeable difference in decrease or increase of number of tillers, the effect of other external factors was similar to that at Lyallpur.

Results obtained from sowings made with the local plough at different dates, depths and spaces, etc., were similar to those stated above.

B. *Rates of Tiller Formation.*—During the course of this investigation the number of tillers per plant were recorded weekly both at Pusa and Lyallpur. The data recorded at Pusa have been given in detail by Pal and Alam (1938). The data for the year 1934–35 are given below in Table II.

It is evident that at the beginning and also near the cessation of tiller formation the rate is comparatively less. Moreover in every sowing there is some period during which the rate of tiller formation abnormally accelerates. For instance in 15/10 and 22/10/34 sowings it is from 24/12 to 7/1 while in 29/10 and 12/11 it is 21/1 to 28/1, etc., etc. This was observed in all the sowings made at various depths and spaces.

(i) *Effect of sowing at different dates.*—From the total number of tillers per plant it was seen that in early sowings and especially in October ones the number of tillers was the highest. But from the constants worked out with the help of exponential curves it was revealed that the rate of tiller formation was highest in November sowings and thereafter as the sowings were delayed

TABLE II

Weekly comparison of number of tillers per plant of the variety Punjab 8 A when sowings were made on different dates

Sowing dates	Weekly counts																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
15–10–34 . .	1·5	2·0	2·9	4·1	5·2	7·5	9·4	12·2	16·4	19·2	21·9	22·2	25·4	26·0	26·1	26·3	26·2
22–10–34 . .	1·8	3·0	4·2	6·2	7·8	10·6	14·5	18·4	20·0	20·1	21·6	23·8	24·2	25·4	25·2		
29–10–34 . .	1·4	2·4	3·2	5·0	5·8	6·7	8·2	9·9	11·8	12·9	16·2	18·4	20·7	22·8	23·5	23·5	
5–11–34 . .	1·1	1·8	3·4	4·1	5·4	5·9	6·8	7·2	9·4	13·2	14·4	16·2	18·4	19·2	19·3	20·2	20·2
12–11–34 . .	1·5	2·0	2·2	4·2	5·0	5·8	6·9	10·4	11·4	13·8	14·6	17·8	18·1	18·1			
19–11–34 . .	1·2	2·0	2·4	3·0	3·8	4·0	4·2	5·8	10·4	11·1	12·2	13·0	13·0	13·0			
26–11–34 . .	1·3	1·5	2·2	3·0	3·7	3·9	4·1	8·9	9·0	9·3	11·1	11·1	11·2	11·2			
3–12–34 . .	1·1	1·5	1·8	2·1	3·0	3·4	7·2	8·1	8·7	9·2	9·3	9·4	9·4				
10–12–34 . .	1·3	1·6	2·0	2·8	4·9	5·1	5·2	6·6	6·8	7·0	7·1	7·1					
17–12–34 . .	1·2	1·9	2·1	3·2	3·9	4·8	4·8	4·9	5·1	5·1	5·2						
24–12–34 . .	1·8	2·1	2·5	2·9	3·0	3·2	3·5	3·8	4·0	4·1							
31–12–34 . .	1·1	1·3	1·8	2·2	2·5	2·8	2·9	3·1	3·2								
7– 1–35 . .	1·1	1·4	1·8	2·1	2·4	2·5	2·8	2·9									
14– 1–35 . .	1·2	1·6	1·8	2·3	2·5	2·6	2·7										
21– 1–35 . .	1·1	1·5	2·1	2·4	2·5	2·6											

the rate went on decreasing. It appears that the November sown plants are the most efficient in tiller production when the latter is considered per unit of time.

(ii) *Effect of other external factors.*—At wider spacings and shallower depths the efficiency of tiller formation was more than at narrower spaces and deeper depths. During the year 1933–34 the number of tillers being comparatively greater the efficiency was also greater. Similarly at Pusa the number of tillers was practically the same in both the years and hence there was not much difference in rate of tiller formation.

C. *Number of Effective Tillers.*—The tillers with and without ears were counted and the data recorded were subjected to statistical test. The effect of sowing dates was the greatest, being followed by spacings, that of depths was the least. Data for the year 1934–35 are given below in Table III.

TABLE III

Percentage of ears to tillers in Punjab 8 A *wheat sown at different spaces, depths and dates*

Spacing from plant to plant	Sowing depth	Sowing dates (1934–35)								
		15/10	19/11	3/12	10/12	17/12	24/12	31/12	14/1	21/1
3″	1″	97·8	90·0	74·1	64·0	58·1	49·1	46·0	24·1	14·7
	3″	95·2	89·2	66·0	58·0	51·2	43·1	41·2	19·2	12·0
	5″	92·1	85·2	62·1	52·2	47·1	37·1	32·4	14·3	8·2
3″	2″	96·9	91·1	72·1	64·2	52·1	46·1	45·0	22·1	13·8
9″		90·2	89·2	64·2	55·5	39·1	41·1	36·0	12·6	8·4
15″		90·3	84·4	60·2	49·4	42·0	37·1	29·1	9·4	5·1

(i) *Effect of sowing at different dates.*—It is apparent that sowing dates have caused a great influence on the percentage of tiller bearing ears. The crop sown in the beginning of December produces only 75 per cent. of effective tillers, that sown in the second half of December produces 50 per cent., while that sown in January produces only 15 per cent.

(ii) *Effect of other external factors.*—It has been seen that in the case of first depths, *i.e.*, up to 3″, the differences up to 24/12 remain insignificant. This later on comes down to first two depths only.

Similarly in case of sapcings after 19/11 the difference between first two spaces *i.e.*, 3″ and 6″ remains insignificant only upto 24/11. Thereafter it is the 3-inch spacing only which gives the highest percentage of ears to tillers.

This leads to the conclusion that in late sowings it is advisable to sow at shallow depths and at narrow spacings and further that as compared with early sowings, a higher seed-rate should be used.

Similarly conclusions were arrived at from other experiments carried out at Lyallpur and Pusa.

6. *Height*

In all the four years measurements of the main stem were recorded weekly. Statistical analysis showed that the great effect in all the experiments had been caused by sowing dates. It was followed by spacings and then by sowing depths. Interactions only between dates and depths, and dates aud spacings were significant. The data are given in Table IV below:

TABLE IV

Comparison of height of main stem in cm. of Punjab 8 A *wheat sown at different depths, spaces and dates* (1933–35)

Spacing from plant to plant	Sowing depth	Sowing dates (1933–35)									
		15/10	29"10	5/11	19/11	26/11	3/12	10/12	24/12	31/12	28/1
12"×12"	1"	148	154	140	130	120	106	96	80	56	56
	3"	144	149	134	128	119	104	96	78	66	50
	5"	138	144	128	113	95	84	80	62	54	38
		1934–35									
		29/10	12/11	26/11	3/12	10/12	24/12	31/12	21/1		
9"×3"	1"	154	146	140	128	119	109	102	92		
	3"	151	145	141	124	118	106	96	87		
	5"	146	138	132	116	108	96	86	76		
2"	3"	153	144	139	126	117	108	101	91		
	9"	152	141	136	114	110	105	88	79		
	15"	144	132	124	110	102	88	79	68		

Variety	1935–36	1936–37			
	29/10	29/10	12/11	26/11	10/12
Pusa 52	101	92	88	89	76
Pusa 165	97	89	86	91	81

A. *Total Heights*—(i) *Effect of depths.*—There was no effect of sowing depths up to November sowings. Thereafter up to the middle of December the 5-inch depth sowing remained significantly different from other depths. In later sowings the 4-inch depth sowing also becomes significantly different from those at the shallower depths.

(ii) *Effect of spacing.*—In October sowings there was no significant effect of spacing on height though the tendency was that at wider spaces the height became less. In November sowings the height of plants sown 15 inches apart was significantly less than that of plants sown 3 inches apart. Thus further it is seen that in December sowings height of 12-inch spaced plants also becomes less and in January sowings the difference between only 3-inch and 6-inch apart plants remains insignificant.

(iii) *Effect of season and sowing on different dates on the height of plant.*– Average height under different treatments was correlated with sowing dates. The correlation co-efficients were very high, negative and significant. The plants with least height were obtained in January sowings. An interesting feature observed was that in certain cases the difference in height was very much marked while in others it was not so appreciable. For instance in 1933-34 the difference between heights of 29/10 and 5/11, 19/11 and 26/11, 26/11 and 3/12, 3/12 and 10/12, 24/12 and 31/12 looked to be very much marked while the differences between that of 12/11 and 19/11, 17/12 and 24/12 or 7/1, 14/1 and 21/1 or 28/1, appeared to be practically nil. These differences being of very outstanding nature were next year observed still more keenly. It was seen that next year there was not much difference between 5/11 and 12/11, 19/11 and 26/11, 17/12 and 24/12, or 7/1, 14/1 and 21/1. This shows the effect of season.

What has been said above is based on the height of the main stem, but it was observed that in case of late sowings and specially after 24/12 there were hardly one or two tall tillers and the others were extremely dwarf. Thus if average heights of all the tillers were compared, the height of December and January sowings would have been much less as compared with what has been given above. The data collected in this respect at Pusa are given below. The figures show percentage increase of height of main stem over the average height of all the tillers :—

Variety	1935–36	1936–37			
	29th Oct. sowing	29th Oct. sowing	12th Nov. sowing	26th Nov. sowing	10th Dec. sowing
Pusa 52	8·5	9·8	19·8	19·6	23·6
Pusa 165	6·7	10·2	17·8	17·2	20·0

B. *Rate of Growth of Height of Main Stem.*—(i) *Effect of sowing on different dates on rate of growth of height.*—The results of weekly measurements are graphically represented in Fig. 2. It is evident that growth of wheat plant has three peculiarities. In the beginning it makes very rapid

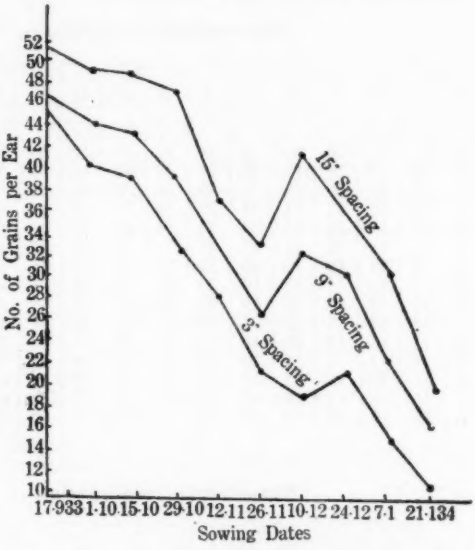

FIG. 2

Illustrating the comparative number of grains per ear of the Punjab 8 A wheat (1933-34)

growth in height both in erect and prostrate varieties. Then the development is restricted to basal leaves and tiller formation only, *i.e.*, it makes practically no growth in height. After some time it shoots and abruptly begins to grow at an accelerated speed. Of course for sometime tiller formation also goes on but ultimately it stops and plant is left to grow in height only. Near heading it makes another jump and thereafter growth is retarded and ultimately ceases when the crop nears ripening.

For working out the relative growth rate the data were taken from germination to the cessation of growth in height as well as for the active period alone which began from jointing. In general the values for the total period should have been less than for the active period alone but in case of October and November sowings the reverse was the case, which brings out that the growth was more rapid before than after jointing. In January sowings the growth rates of both the periods were practically the same.

TABLE V

Illustrating the comparative height of plant of the Punjab 8 A wheat (in c.m.) when sowings were made on 15 different dates during the year 1934–35 at Lyallpur

Sowing dates	Measurements—weekly counts																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
15–10–34 ..	9	14	16	18	20	23	24	28	32	37	42	45	50	56	63	68	74
22–10–34 ..	8	12	14	15	16	18	27	28	34	40	44	51	55	64	69	72	80
29–10–34 ..	7	12	16	18	20	22	24	30	34	38	44	51	59	66	70	78	93
5–11–34 ..	6	11	12	15	17	21	24	29	34	37	40	44	48	51	59	67	82
12–11–34 ..	5	10	14	19	21	25	27	32	38	40	45	49	54	61	68	80	96
19–11–34 ..	5	9	15	18	19	21	21	22	23	26	31	37	45	54	65	80	97
26–11–34 ..	5	8	12	15	18	19	20	22	25	32	37	44	50	61	78	94	110
3–12–34 ..	6	10	11	13	14	15	19	20	21	23	28	36	44	58	70	86	99
10–12–34 ..	6	10	13	16	17	18	19	20	23	26	34	39	51	65	80	94	103
17–12–34 ..	5	11	15	17	19	20	22	23	25	27	32	48	62	76	91	100	106
24–12–34 ..	6	10	12	13	14	16	16	18	21	26	33	42	56	72	84	89	92
31–12–34 ..	5	9	11	12	13	15	16	18	21	25	35	47	58	67	75	80	83
7– 1–35 ..	5	8	11	14	15	15	17	20	22	34	45	55	65	70	75	78	80
14– 1–35 ..	4	7	11	12	13	16	17	20	23	34	47	54	62	68	72	74	
21– 1–35 ..	4	8	11	15	16	17	19	21	31	40	51	60	65	68	70		

Sowing dates	Measurements—weekly counts													
	18	19	20	21	22	23	24	25	26	27	28	29	30	31
15–10–34 ..	82	98	110	121	129	136	142	146	150	151	152	151		
22–10–34 ..	96	111	120	128	134	141	147	151	152	153	153			
29–10–34 ..	109	118	124	130	142	141	152	153	152					
5–11–34 ..	100	112	124	132	141	146	143	148	146					
12–11–34 ..	111	121	132	140	144	146	147	145						
19–11–34 ..	112	126	132	137	138	140	139							
26–11–34 ..	127	133	136	139	141	141								
3–12–34 ..	109	116	122	124										
10–12–34 ..	108	112	115											
17–12–34 ..	108	109	110											
24–12–34 ..	95	96												
31–12–34 ..	84													
7– 1–35 ..														
14– 1–35 ..														
21– 1–35 ..														

Another interesting point brought out was that in terms of main stem production per unit time the October sowings were the least efficient while January ones were the most efficient. The December sowings were the second best and were followed by November sowings. Should the conclusions have been based on average height of all the tillers, these in all probability would have been different from what have been arrived at above.

(*ii*) *Effect of other external factors.*—At greater depths and wider spaces the rates were comparatively low, otherwise the trend on different dates was the same. Season did not have any noticeable effect on growth rate as measured by increase in height.

7. *Emergence and Ripening of Ears*

(i) *Effect of sowing at different dates and season.*—Both at Lyallpur and Pusa it was observed that date of sowing affects the time of emergence and of ripening of ears. Season had no noticeable effect on the ear characters. The results of the effects of sowing at different dates are rather striking and are given below for Punjab 8 A wheat grown during the year 1934–35:—

No. of days from	Sowing dates									
	17/9	1/10	15/10	29/10	12/11	26/11	10/12	24/12	7/1	21/1
Sowing to emergence ..	140	131	124	117	110	103	97	88	83	80
Sowing to ripening ..	203	192	180	167	158	150	140	133	125	113
Emergence to ripening ..	63	61	56	50	48	47	43	45	42	33
1st sowing to next sowing	..	14	28	42	56	70	84	98	112	126

It is evident that difference between seeding dates of any two sowings is throughout more than the difference between emergence and ripening. Similarly difference between emergence of different sowings is always less than the difference between ripening of different sowings. It has been seen that it is not that any definite duration, *i.e.*, number of days are required for emergence or ripening of the crop, but there is a time bar after which the crop matures and dries. For instance all January sowings ripened on or about 13–5–35 though the difference between sowing dates varied from 7 to 30 days.

(ii) *Effect of other external factors.*—The difference in case of earing and ripening between 1-inch and 5-inch depths was 4 to 7 days in November sowings, 8 to 11 days in December ones and 3 to 5 days in January ones. Similarly the differences between 5-inch and 15-inch spacings from plant to plant were 3 to 8 days, 10 to 13 days and 4 to 6 days respectively. It took always longer to ear as well as ripen at greater depths and spaces.

Conclusions arrived at from other experiments carried out at Pusa and Lyallpur did not vary materially from what has been said above.

8. *Length and Thickness of Ear*

Length of ear taken to mean the length of rachis from the base of the lowermost spikelet whether fertile or sterile to the top of the flowering glume on the terminal spikelet, excluding the awn, was measured in all the experiments both at Pusa and Lyallpur. When measuring it was found that thickness of the ear was about 1/10th of the length when it was between 6 and 10 cm., when it was below 6 cm. thickness was about ·05 cm. more than 1/10th of the length, and when it was longer than 10 cm. the reverse was the case.

(i) *Effect of sowing at different dates and season on length of ear.*—Season caused practically no difference in ear length both at Lyallpur and Pusa. But the dates of sowing had a very complicated effect in conjunction with other external factors such as depth and space. It will be clear from the following data:—

(*Sowing dates* 1934–35 (*Lyallpur*)

Ear length in cm.

Spacing from plant to plant	Sowing depth	Sowing dates (1934–35)								
		1/10	15/10	29/10	12/11	26/11	10/12	24/12	7/1	21/1
3″	2″	8·9	8·6	8·8	8·0	7·6	6·8	6·4	6·2	6·1
9″		9·6	9·4	9·4	8·3	9·2	9·9	10·0	9·4	9·6
15″		10·3	10·4	9·9	8·9	10·9	12·2	12·0	10·6	10·8
3″	1½″	9·0	9·0	8·9	8·4	7·9	6·5	6·1	6·0	5·8
	5″	8·9	9·0	8·8	8·2	7·4	6·1	5·9	5·5	5·5

Pusa

	1935–36	1936–37				
	29/10	29/10	12/11	26/11	10/12	
Pusa 165 Main ear ..	11·0	11·3	11·4	11·5	10·1	Spacing from plant to plant in both the years was 12″×6″
Do. mean of all the ears ..	9·5	9·5	10·0	10·2	9·6	
Pusa 52 Main ear ..	9·9	9·2	9·7	8·5	8·2	
Do. mean of all the ears ..	8·4	8·1	8·0	8·2	8·0	

(ii) *Effects of depths and spacing.*—It is very interesting to note that at wider spacing in November and December sowings the length of ear increased from 8·9 cm. to 12·0 cm., while on the other hand in closer spacing it decreased from 8·0 cms. to 6·4 cms. Again in January sowings the length decreased even in widely spaced plants.

(1) At Pusa the results from sowings made on 30th of October and 10th of December 1936 were:—

Spacing from plant to plant

	30th October sowing			10th December sowing		
	3″	9″	15″	3″	9″	15″
Pusa 52	8·3	8·5	8·6	6·4	7·9	8·2
Pusa 165	9·1	10·1	10·5	7·1	8·2	9·6

Up to the end of October there seemed to be no appreciable effect of different depths. But as the sowings were delayed the effect went on increasing and in the last sowings it became very much marked.

(2) The results for Pusa wheats sown on 12–12–36 are:—

	Sowing depths		
	1″	3″	5″
Pusa 52	8·0	7·7	7·8
Pusa 165	9·5	8·6	9·1

The results obtained from other experiments were in conformity with what has been said above.

(iii) *Association between ear length and height of tiller.*—Within the plant a very high and positive correlation was found. But when different plants grown under similar conditions were examined, although the correlation was still positive but not so high. In plants from different treatments there was no association at all. Similarly correlations between length of ear stalk, number of internodes, leaves per tiller and number of tillers, both effective and total per plant, was studied and it was found that there was no association between these characters and ear length.

9. *Number of Fertile and Sterile Spikelets*

The number of spikelets were effected considerably by date of seeding and the spacing from plant to plant. The data are summarised below:—

Sowing dates (1934–35, *Punjab 8 A Lyallpur*)

Spacing from plant to plant	Sowing dates									
	17/9	1/10	15/10	29/10	12/11	26/11	10/12	24/12	7/1	21/1
3″ Fertile	20·0	18·8	19·3	17·9	17·2	14·9	13·9	12·9	9·8	7·8
Sterile	·8	1·1	1·9	1·9	3·8	3·5	1·2	1·5	4·6	5·2
9″ Fertile	21·0	20·4	20·8	19·8	18·8	17·9	16·2	17·4	13·2	12·5
Sterile	·7	1·1	1·3	2·2	3·5	2·9	1·4	1·4	5·8	6·1
15″ Fertile	22·8	22·3	21·9	20·9	19·4	19·8	18·9	19·2	15·2	13·2
Sterile	·9	1·5	1·0	2·0	2·8	2·4	1·2	1·7	5·3	6·7

Pusa (1935–37)

	1935–36		1936–37		
	29/10	29/10	12/11	26/11	10/12
Pusa 165 Fertile	17·6	15·3	15·9	15·9	14·1
Sterile	2·2	2·9	2·8	2·9	3·1
Pusa 52 Fertile	17·1	17·7	19·0	17·0	16·4
Sterile	2·3	2·0	2·5	2·7	3·6

A. *Fertile Spikelets*—(i) *Effect of season and date of sowing.*—Season did not have much effect on number of fertile spikelets per ear. But date of sowing had a conspicuous effect on this character. The number of fertile spikelets in January sowings decreased to about 50 per cent. of that observed in the early sowings.

(ii) *Effect of spacings and depths.*—At wider spacings ear length increased in December sowings (*supra*) but the number of spikelets decreased with the result that the ears become more lax and the size of spikelets also became greater. In closely spaced plants however the corresponding decrease in number of spikelets did not take place and hence the density does not go down and even in some cases it increases. In plants at wider spacing the number of fertile spikelets was throughout greater than in closely spaced ones.

The results from Pusa wheats sown on two different dates are :—

	Spacing from plant to plant					
	30th October sowing			10th December sowing		
	3″	9″	15″	3″	9″	15″
Pusa 52 Fertile ..	16·1	17·1	17·1	11·1	16·4	17·2
Sterile ..	2·2	2·1	1·8	2·7	2·8	2·7
Pusa 165 Fertile ..	13·8	14·6	14·7	10·6	13·5	15·3
Sterile ..	2·3	2·4	2·6	2·4	2·3	3·1

It is obvious that the varieties have responded very differently to this factor.

Sowing depths did not have any noticeable effect, though in late sowings at shallower depths the number was comparatively greater.

B. *Sterile spikelets*—(i) *Effect of season and date of sowing.*—It was seen that in general the number of sterile spikelets was more in late sowings both at Lyallpur and Pusa. The only exception found was that in December sowings at Lyallpur the number was comparatively less while the reverse was observed at Pusa.

It was seen that top spikelets were very seldom sterile whereas the basal ones were usually sterile.

(ii) *Effect of spaces and depths.*—The percentage of sterile spikelets was much less at wide spacings and shallow depths. Under all the conditions, well developed and especially the first-formed tillers had comparatively lesser numbers of sterile spikelets.

Season did not have much effect on this character.

Other experiments carried out both at Lyallpur and Pusa gave similar results.

10. *Number of Grains per Ear*

All grains which were formed irrespective of their being well developed or not were counted. The results for Punjab 8 A grown during the year 1934–35 are shown in Fig. 3. It is evident that date of seeding affects the number of grains per ear to a great extent. In September and October sowings in close and wide spacings the number is about 45 to 50; it comes down in November sowings to 28 to 31, in December ones to 19 to 41 and in January ones 11–21. From December onward the number of grains at

closely spaced sowings goes down considerably as compared with wider spacings. Similarly greater sowing depths in December and onwards show more marked effect than in early sowings.

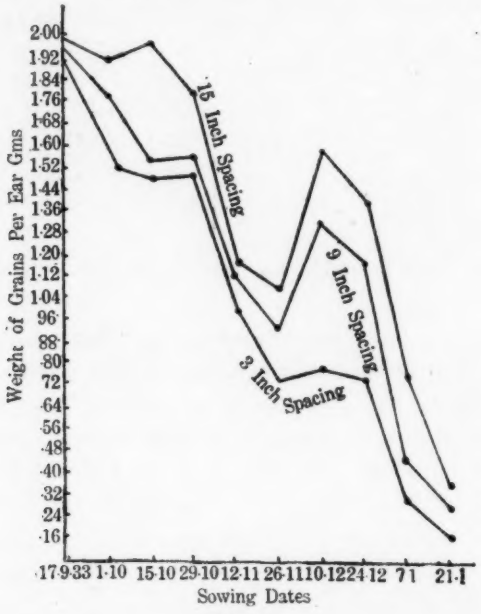

FIG. 3

Illustrating the comparative weight of grain per ear of Pnnjab 8 A Wheat (1933-34)

The results obtained at Pusa and Lyallpur in some experiments are rather striking and are given below:—

Variety	No. of grains per ear				
	1935–36	1936–37			
	29th Oct. sowing	29th Oct. sowing	12th Nov. sowing	26th Nov. sowing	10th Dec. sowing
Pusa 165	33·0	26·0	26·0	23·5	21·8
Pusa 52	40·6	32·0	26·8	28·0	25·7

Seed rate per acre of Punjab 8 A (1934–35)

	8 seers	16 seers	24 seers	32 seers
Number of grains per ear ..	35·2	33·4	28·3	26·1

This character, *i.e.*, number of grains per ear has been most affected, by all the external factors even including season. It appears that under favourable conditions the number of grains per ear or per spikelet becomes more as compared with other ear characters.

Wider spacings especially in December sowings gave more number of grains per spikelet than close spacings. In all the sowings the spikelets from the top portion of every ear had the least number of grains, these were followed by the basal ones; the middle ones had the greatest number.

11. *Size of Grain*

Wheat is one of the staple crops and if the grain is bold its value is enhanced in the eyes of both consumers and dealers. Length, breadth and thickness of grain were measured, and the results, etc., are summarised below:—

Sowing dates (1934–35, *Punjab* 8 A *Wheat*, *Lyallpur*)

Grain dimensions in millimeters	Spacing from plant to plant	Sowing dates								
		1/10	15/10	29/10	12/11	26/11	10/12	24/12	7/1	21/1
Length	3″	6·32	6·38	6·37	6·02	5·91	5·82	5·52	5·32	5·14
	15″	6·41	6·40	6·39	6·15	6·04	6·68	6·57	6·68	6·08
Breadth	3″	2·83	2·81	2·76	2·69	2·51	2·49	2·32	2·31	2·02
	15″	2·88	2·87	2·84	2·76	2·59	2·88	2·86	2·46	2·19
Thickness ..	3″	2·64	2·63	2·59	2·48	2·40	2·25	2·21	1·81	1·64
	15″	2·69	2·65	2·65	2·54	2·46	2·37	2·28	1·96	1·72
	Sowing depth									
Length	1½″	6·35	6·34	6·26	6·14	6·04	5·91	5·41	5·21	5·08
	5″	6·38	6·32	6·24	6·11	6·00	5·29	5·01	5·09	4·99
Breadth	1½″	2·83	2·78	2·77	2·69	2·49	2·40	2·36	2·38	1·96
	5″	2·81	2·76	2·72	2·62	2·44	2·39	2·30	2·35	1·87
Thickness ..	1½″	2·67	2·65	2·61	2·50	2·39	2·28	2·19	1·75	1·57
	5″	2·65	2·63	2·62	2·49	2·36	2·25	2·12	1·70	1·64

Pusa wheats (1935–37, *Pusa*)

		1935–36	1936–37			
		29/10	29/10	12/11	26/11	10/1
Pusa 165	Length	5·96	6·29	6·12	6·20	5·58
	Breadth	3·00	3·19	3·29	2·77	2·37
	Thickness	2·51	2·71	2·79	2·26	2·17
Pusa 52	Length	5·45	5·61	5·61	5·67	5·29
	Breadth	2·74	2·55	3·19	2·63	2·24
	Thickness	2·43	2·79	2·82	2·35	2·02

(i) *Effect of sowing at different spacings.*—The size and shape of grains were obviously affected by sowing at different spaces. At wider spacings the grains were more bold and especially in late sowings.

(ii) *Effect of sowing at different depths.*—All the three variables, *i.e.*, length, breadth and thickness were not much effected in early sowings on account of planting at different depths. In late sowings the greater depths adversely affected the size and shape of grain.

(iii) *Effect of season and date of sowing.*—The season had practically no effect on size and shape of grain. Date of sowing up to end of October had very little effect on all the three variables and in fact on the basis of these three factors the best quality of grains was obtained from crops grown in September and October.

From October onward thickness of grains went on decreasing. Length and breadth in early December sowings made at wider spacings increased to some extent and thereafter these also began to decrease. The grains obtained from December and January sowings were badly shrivelled.

12. *Grain Weight*

Grain weight is another character which responds strikingly to all the external factors studied.

(i) *Effect of sowing at different dates and season.*—Date of sowing affects the grain weight considerably. At Lyallpur weight of Punjab 8 A wheat per 100 grains was 3·89 grams in September and October sowings; in November it was 3·42 and in January it was only 1·89 grams. However there was an exception and that was that at wider spacings instead of decreasing the weight increased from 3·42 to 3·94 grams which afterwards again began to decrease. Fig. 3 displays the results.

At Pusa, weight per 100 grains in grams on different dates was :—

	29th Oct. sowing	12th Nov. sowing	26th Nov. sowing	12th Dec. sowing
Pusa 165	3·8	3·05	2·6	2·3
Pusa 52	3·15	2·6	2·58	2·3

The effect of sowing at different dates and the differential response of these varieties to this factor is obvious.

Season affected the grain weight to some extent but its influence was not so marked.

(ii) *Effect of other external factors.*—Sowing depths did not affect the grain weight considerably except in late sowings.

Spacing as mentioned *supra* affects the number of spikelets per ear, spike length and number of grains per ear. For these reasons it also affects the grain weight per ear. It was seen that weight of 100 grains was more at wider spacings as compared with closer spacings. The effect of wider spacings becomes more marked in December sowings when weight at wider spaces increases while at closer spaces it decreases.

Similar results were obtained from other experiments conducted both at Lyallpur and Pusa.

13. *Quality of Grain and Straw*

(i) *Effect of sowing at different dates.*—For judging the quality of grain and straw the percentage of protein and ash were found in case of wheat grown at Lyallpur. The figures of nitrogen and ash percentages are given below :—

		17/9	1/10	15/10	29/10	12/11	26/11	10/12	24/12	7/1	21/1
Nitrogen percentage	Grain ..	1·84	1·99	2·15	2·01	2·00	2·24	2·42	2·55	2·68	3·18
	Straw ..	·621	·551	·681	·571	·564	·468	·485	·491	·620	·641
Ash percentage	Grain ..	1·91	1·84	1·72	1·71	1·69	2·01	2·26	2·35	2·84	2·98
	Straw ..	9·48	9·69	9·64	9·31	9·18	9·38	9·69	9·82	11·24	12·31

It is obvious that as cold season advances percentages of nitrogen and ash increase. But it may be remembered that total quantity per acre will have to be judged from yield per acre and not merely from nitrogen and ash percentages.

(ii) *Effect of other external factors.*—It was observed that season and soil had a great effect on these percentages. Sowing depths, however, did not have any appreciable effect. In September to November sowings at the wider spacings the nitrogen and ash percentages were higher than in closely spaced sowings, but this difference became negligible in the December and January sowings.

14. *Grain Flinty or Mealy*

Grains were cut transversely by a cutter and were examined as to their texture—flinty, semi-flinty or mealy. The grains which were dense and translucent were classified as flinty, those which possessed white, opaque and

chalky endosperm were classified as mealy, while grains which were partly translucent were classified semi-flinty.

(i) *Effect of sowing at different dates and season.*—The following data show the effect of different dates :—

Date of sowing	Produce		
	Flinty %	Semi-flinty %	Mealy %
19- 9-34 to 8-10-34	90	6	4
15-10-34 to 29-10-34	86	8	6
5-11-34 to 26-11-34	76	14	10
3-12-34 to 10-12-34	86	14	..
17-12-34 to 28- 1-35	100	..	..

Season did not have any noticeable effect on this character of grains. This character was not studied at Pusa.

(ii) *Effect of spaces and depths.*—Differences were observed only in sowings made up to the end of November. In December and January sowings there was no effect of spaces and depths. When sowings were made at different spaces the results were :—

Sowing dates	Sowing Spaces								
	3″			9″			15″		
	Flinty %	Semi-flinty %	Mealy %	Flinty %	Semi-flinty %	Mealy %	Flinty %	Semi-flinty %	Mealy %
17- 9-34 to 8-10-34 ..	84	3	13	89	5	6	98	2	..
15-10-34 to 29-10-34 ..	92	8	..	98	2	..	100	..	..
5-11-34 to 26-11-34 ..	72	12	16	82	8	10	90	8	2

When sowings were made at different depths the results were :—

Sowing depths	Flinty %	Semi-flinty %	Mealy %
1″	80	6	14
3″	76	9	15
5″	64	8	28

These results are for November sowing only, in other months the differences were not noticeable.

15. *Yield*

As already stated sowings of Punjab 8 A were done on five different dates to study the effect of the external factors on yield. The results obtained are given in Table VI : --

TABLE VI

Average yield in maunds per acre of the Punjab 8 A wheat when sowings were made on different dates, spaces and depths

Sowing depths	Spacing from plant to plant	Sowing dates (1934-35)									
		15/10		29/10		17/11		1/12		23/12	
		Total	Grain	Total	Grain	Total	Grain	Total	Grain	Total	Grain
		Maunds per acre									
2½″	3″	90·1	24·7	103·7	29·3	87·2	20·0	80·2	7·5	50·5	9·1
	9″	86·2	25·0	92·8	27·2	70·3	15·4	60·0	10·6	32·5	6·2
	15″	86·7	24·4	89·3	25·9	65·3	10·2	34·2	5·9	20·8	3·6
5″	3″	73·4	19·9	91·0	23·1	57·2	13·7	48·3	8·4	31·6	5·1
	9″	72·4	18·8	84·2	22·0	51·9	11·6	31·6	5·6	15·7	2·4
	15″	68·4	17·2	76·4	20·1	45·5	7·9	18·2	3·0	8·5	1·2

The statistical analysis of these data showed that the mean squares contributed by the sowing dates was the largest. The effect of all other components except that for the interaction between the depths and the spaces was significant.

(i) *Effect of sowing at different depths on yield.*—The differences between sowings made at 1½″ and 2½″ depths were insignificant except in the last sowing in which the yield were in favour of the former. Under all the conditions the 5-inch depth yielded the least. As the sowings were delayed the percentage difference went on increasing. In case of 15th October sowing the percentage difference in grain yield between 2½″ and 5″ depths is about 20 per cent. while in case of 23rd December sowing it comes to 44 per cent. This leads to the conclusion that in late sowings it is comparatively more advantageous to sow wheat at shallower depths.

In case of sowing made on 17th November 1934 at three different depths the results are:—

	Sowing depths		
	1½″	2½″	5″
Total yield in maunds per acre	90·75	90·84	73·5
Grain yield in maunds per acre	19·82	19·63	13·98

These results confirm what has been said above. Similar results were obtained at Pusa also.

(ii) *Effect of sowing at different spacings on yield.*—The yield differences in case of 15th October sowing except between 3-inch and 15-inch spacing at 5-inch depth are insignificant. In the case of second sowing which was made on 29th October the differences between 3-inch and 15-inch were throughout significant while between 3-inch and 9-inch only at 1½-inch depth the difference was significant. In the next three sowings which were made on 17th November, 1st December and 23rd December the differences between 9-inch and 15-inch spacing were not remarkable. But, however, the differences between 3-inch and 15-inch, and 3-inch and 9-inch spacings were significant.

In the case of another experiment where sowings were made at three different spaces on 17th November the yields obtained are:—

	Spacing from plant to plant		
	3″	9″	15″
Total yield in maunds per acre ..	86·3	79·5	69·8
Grain yield in maunds per acre ..	18·5	14·7	11·9

A similar experiment was conducted at Pusa and the results are:—

	Spacing from plant to plant		
	3″	9″	15″
Grain yield per unit area in grams of :—			
Pusa 165	2·25	2·40	2·42
Pusa 52	2·9	3·51	3·95

(iii) *Effect of sowing on different dates on yield.*—From Table VI, it is evident that up to end of October as the sowing is delayed the yield goes on increasing. The sowing which was done in the end of October has given the highest yield.

Similar results which were obtained at Pusa are given below:—

Year 1936—37

Yield per plant in grams

	1st sowing 29/10	2nd sowing 12/11	3rd sowing 26/11	4th sowing 10/12
Pusa 52	12·2	9·2	8·8	2·5
Pusa 165	11·0	7·3	6·6	2·1

During the year 1933-34 an early and late sowing of Punjab 8 A wheat was done on a field scale and results similar to those mentioned above were obtained.

In another experiment at Lyallpur three varieties were compared in randomised blocks with eight replications. The size of each sub-plot was 1/10th of an acre. The results are:—

	Sowing dates	
	30-10-34	6-12-34
	Maunds per acre	
8A	27·05	18·22
9D	22·93	18·84
C591	27·03	20·80

Another experiment was conducted at the Multan Agricultural Station during the year 1935-36 and the results are:—

	Sowing dates (1935-36)		
	16-10-35	31-10-35	16-12-35
	Maunds per acre		
8A	14·5	12·5	8·9
9D	13·0	12·1	8·3
C591	11·8	10·4	7·8

From the data given above the effect of varieties, sowing dates and interaction between sowing dates and varieties is quite obvious.

From what has been said above, it can safely be concluded that it may be misleading to compare varieties by sowing them on only one date. It is possible that many varieties have been rejected in varietal trials as a result of comparing them under one set of conditions only ; under a slightly different set of conditions, different results might have been obtained.

IV. Summary

1. Developmental studies were made on three varieties of *Triticum vulgare, viz.*, Punjab 8 A at Lyallpur and Pusa 52 and Pusa 165 at Pusa, under different dates and times of sowing, different depths of sowing, different condition of spacing and different soils. Trials under different soils were made only at Lyallpur.

2. The optimum range of sowing depths for Punjab 8 A wheat was found to be 1–3½″ in light loam, 1–3″ in loam and 1–2½″ in heavy loam soil. Season was found to influence the optimum range. In early sowings it varied from 1½–3″ and in medium 1–3″ and in late ½–3″. For Pusa varieties it was found to be 1–3″.

3. Time and depth of sowing and season considerably affected the rate of germination. At greater depths and in low temperatures it was very slow. Irrespective of date, time and depth of sowing and the soil, the rate of germination was very rapid from midnight to 8 A.M., while it was very slow from noon to midnight. The time for start and completion of germination was longer when the mean daily temperature was low. The number of days for start and completion of germination were 4 and 6 in October sowings and 18½ and 29 in January ones.

4. Spacing from plant to plant did not affect the mortality of seedlings. Date of sowing had a considerable affect on survival percentage, it was 1·1, 2·8, 24·1, 37·2, 57·4, 90·8, 92·2 and 97·2 in case of sowing made on 17th September, 24th September, 1st October, 8th October, 15th October, 22nd October, 29th October, 5th November and 12th November respectively. In sowings made after 12th November no mortality took place. At shallow depths in early sowings mortality was comparatively greater.

5. Variation in respect of the rate of formation of the first four foliage leaves and the first tiller was effected by all the factors under study except spacing from plant to plant. Range of time of appearance increased with delay in sowing and greater depths. The number of days to start tiller formation varied from 17 to 45 days. In early sowings it was less.

6. Late sowings tended to decrease the number of leaves produced per tiller, though to a very slight extent. In September to November sowings it was 7–8 and in December to January it varied from 7–5. Other external factors did not cause any appreciable difference.

7. Tiller formation was affected to a considerable extent by all the factors under study. The number of tillers per plant at 3 inch spacing and 2 inch depth varied from 29·4 to 2·8 from October to January sowings. At wider spaces number of tillers per plant was more than at closer ones. But the wider spacings do not show corresponding increase in number of tillers. Moreover if number of tillers produced per unit length is considered the closest spacing appears to be the most effective and this efficiency goes on increasing as the sowings are delayed. At 15-inch spacing in terms of per unit length, the increase over the actual number was 138·1 per cent. in October sowing while it was 253·7 in January sowing.

Sowing depths affected only late sown wheat. Shallow depths did better than deeper ones. It was seen that November sown plants are the most efficient in respect of rate of tiller formation when the latter is considered per unit time.

Date of sowing affected the number of effective tillers considerably. It varied from 97 per cent. in October and November sowing to 14 per cent. in January ones. At wider spacings and greater depths in late sowings the percentage of ear bearing tillers to total number of tillers was comparatively less.

8. There is a very high negative correlation between the date of seeding and height of main stem. The height decreases as the sowings are delayed. It varied from 148 cm. in October sowings to 50 cm. in January ones. Effect of sowing at greater depths and wider spaces became very much more marked in December and January sowings—the height decreased remarkably.

Unlike tiller production the plants are most efficient in the month of January so far as growth of height of main stem is concerned. In fact as the sowings are delayed the efficiency of plants to grow in height goes on increasing.

9. As the sowings are delayed the number of days from sowing to emerging and ripening and from emerging to ripening goes on decreasing. In October the number of days from sowing to ripening and emergence of ears were 192 and 131 while the corresponding figures in January were 125 and 83.

At shallower depths and closer spacings emergence and ripening both were comparatively earlier.

10. At wider spaces the length of ear increases during the months of November and December from 8·9 cm. to 12·0 cm. while on the other hand in closer spacings it decreases from 8·0 cm. to 6·4 cm. In January sowings the length decreases even in case of wide spaced plants.

Effect of depths was only noticeable in late sowings, at shallower depths the length of ear was greater.

11. The number of fertile spikelets goes on decreasing as the sowings are delayed, from 20·8 in October it comes down to 7·8 in January.

At wider spacings the number of spikelets is always greater than closer ones. During the month of December at wider speces the ears become lax because of the increase of ear length while in closer specings they become very dense.

Except in January sowings there is no effect of sowing depths on this character, during this month number of fertile spikelets becomes much less at greater depths as compared with shallower ones.

Sterility in wheat seem to be due to multiplicity of causes such as thick seeding, late sowings and especially at greater depths, and later formation of tillers in individual plants, etc., etc.

12. Date of sowing affects the number of grains per ear to a great extent. In September and October sowings in close and wide spacings the number was 45–50, in November it was 28–37, in December it was 19–41 and in January it came down to 11–21.

In early sowings the effect of depths is not so much marked but in December and January the number becomes much less at greater depths.

In all the sowings the top spikelets of every ear had the least number of grains per spikelet. These were followed by basal ones and the middle ones throughout had the greatest number of grains per spikelet.

13. Date of sowing had very little effect upon size and shape of grain up to the end of october. Afterwards thickness went on decreasing up to the end of January, while length and breadth increased to some extent in early December sowings, and later on these also began to decrease.

In December and January sowings the size and shape of grain were affected adversely at greater depths.

Under all the conditions the grains obtained from wider spaced plants were comparatively more bold.

14. Except December sowings as the sowing is delayed the grain weight per ear as well as per 100 grains goes on decreasing. In December sowings, in close seeding the downward tendency continues but in wider spacings the grain weight increases instead of decreasing.

At wider spacings the grain weight is more than at closer ones.

There was no appreciable effect of sowing depths in early sowing, but in December and January sowings the grain weight was affected at greater depths.

15. As the sowing is delayed the percentage of nitrogen and ash in grain and straw increases.

Sowing depths do not affect the nitrogen and ash contents appreciably. At wider spacings, however, more nitrogen and ash were obtained as compared with closer spaces.

16. In early and late sowings the percentage of flinty grains is more than in November ones, the maximum is in December and January ones.

At greater depths and narrower spacings the percentage of flinty grains was less than at shallower depths and wider spaces.

17. In early sowings the effect of different spacings on the yield is not so marked, but from the beginning of November onward as the sowings are delayed the difference between the narrow and wide spacings goes on increasing. It has definitely been ascertained that thick sowings in general and especially in late sowings do better than thin ones.

18. Whenever sowings are made at greater depths, the yield is affected adversely. But this difference becomes more intensified with delay in sowing. Shallower depths, therefore, are advocated for late sowings.

19. It has been seen that yield of wheat is affected to a great extent by sowings made at different dates in the season. For Lyallpur colony the optimum time for sowing Punjab 8 A wheat has been found to be last week of October and 1st week of November. On a field scale it has been seen that wheat sown on 30/10 yields about 33% more than that sown on 6/12. This difference cannot be made up by sowing the crop at one or the other depth or spacing. It, therefore, appears desirable that the cultivators and the canal authorities should find ways to concentrate their energies in getting the crop sown in the end of October and within a week's time.

20. These studies have brought out a point of an immediate application, *i.e.*, it is a wrong practice to grow the crops under one set of environmental conditions such as by sowing them at one station on only one date.

What should be done is, that the varieties should be tried by sowing them on different dates and stations during the same season. It should be repeated for a number of years to give them different environmental conditions and see under which set of conditions a particular variety does best.

By not doing so there is a danger of discarding the varieties which may not be discarded if given proper trial.

21. The data presented indicate that development of wheat plant is greatly influenced by external factors. Late sowings should preferably be not made and in case they are to be made comparatively higher seed rate should be used and seed should be sown at shallower depths.

The desirability of investigating developmental stages of wheat plant under as wide a range of conoditions as possible has been pointed out.

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POISON GLANDS IN THE PECTORAL SPINES OF TWO CATFISHES—*HETEROPNUESTES FOSSILIS* (BLOCH) AND *PLOTOSUS ARAB* (FORSK), WITH REMARKS ON THE NATURE OF THEIR VENOM

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Introduction

The presence of spines in certain species of fishes capable of inflicting severe wounds has long been known, but the occurrence of poison glands in connection with such spines was disputed by early workers such as Aldrovandus, Lacepede and Cuvier. Alman (1841) was the first to describe the poison gland in the spine of the lesser weever. Porta (1908) pointed out the presence of a glandular structure in the lateral grooves of the poisonous spines of Trygon. Later Evans (1922) has given an excellent account of the anatomy and histology of the poison glands and clinical observations on the action of the venom in *Trygon pastinaca*, *Acanthias vulgaris*, *Cistracion philippi* and *Chimaera monstrosa* (Evans, 1922).

At the suggestion of Prof. A. Subba Rau, D.Sc. (London), F.R.M.S., the study of the poison glands of two species of Indian catfishes—*Heteropnuestes fossilis* (Bloch) and *Plotosus arab* (Forsk) which are reputed to cause poisonous wounds was undertaken. The local name for *H. fossilis* in Mysore is suggestively 'Chelu meenu' which means 'Scorpion fish'. It is a freshwater fish and occurs in India, Ceylon, Burma and Cochin China. *P. arab* is a marine form and is found in the Indian seas. Day (1889) states that "wounds from the pectoral spine of this fish (*H. fossilis*) are dreaded in India, as they are reputed to be very poisonous, even occasioning tetanus. As soon as captured, the offensive spine is broken off by blows with a stake, consequently it is difficult to procure large and perfect specimens. Fishermen dread this fish so much that they would prefer cutting the meshes of their nets and allowing it to escape than endeavour to remove it uninjured," and "Wounds from the pectoral spines of this fish (*P. arab*) are much dreaded by the natives of India and are said to cause severe inflammation or even tetanus." The exact nature of the poison in connection with the pectoral

spines of these fishes is not known. The anatomy and histology of the poison glands in *H. fossilis* and *P. arab* together with some experimental observations on the nature of the venom of the former are recorded in this paper.

Material and Methods

The pectoral spines were cut at the base and fixed in Bouin's fluid and decalcified for 3 to 4 weeks in 70% alcohol with 3% hydrochloric acid. They were then embedded in paraffin and serial sections taken. One set of sections was stained in hæmalum and eosin and another set in Van Gieson's fluid. For the study of the reaction of the venom on frog 250 pectoral spines of *Heteropnuestes* were dried in the sun, powdered and mixed with glycerine. By filtering the mixture about 12 c.c. of the toxic fluid was obtained, which was used for injecting into the frog. For hæmolytic experiments fresh poison was squeezed and dissolved in normal saline solution.

Poison Gland in situ

It is stated (Norman, 1931) that in case of certain catfishes of Northern America (*Noturus* and *Schilbeodes*) the poison gland is in the form of a sac with a, more or less, wide opening at the base of each pectoral spine and it is believed to contain fluid of a venomous nature, which is poured out when the spine is brought into action. Such glands are not present in either *Heteropnuestes* or *Plotosus* as was evident from actual dissection. The occurrence of a different type of poison gland in them was, however, revealed after a microscopic examination of sections of the pectoral spines. The poison is secreted by the basal epithelial cells of the epidermis covering the spine and collects between the skin and the spine. Fig. 1 represents the diagrammatic longitudinal section of the spine showing the poison gland in *Heteropnuestes*. There is no special duct conveying the poison to the outside. When the spine is erected and brought into action, pressure is exerted by the skin on the poison and consequently, it is assumed, the poison is ejected through a temporary rupture of the skin at the tip of the spine into the wound made by the latter.

Histology of the Poison Gland

1. *Heteropnuestes fossilis*.—Fig. 2 represents the transverse section of the middle region of the spine. It is seen that the spine is covered over by the skin. The epidermis is composed of several layers of epithelial cells of which some occur as mucous cells. On either side of the spine are seen large groups of highly enlarged rounded cells which take eosin more prominently than other tissues in the section and are stained yellow in Van

Gieson's fluid ; they constitute the poison glands. A detailed microscopic examination reveals that they appear to be derived from the basal columnar

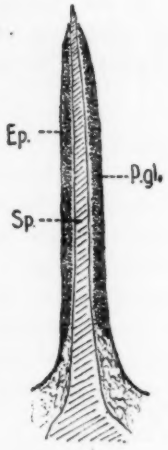

FIG. 1

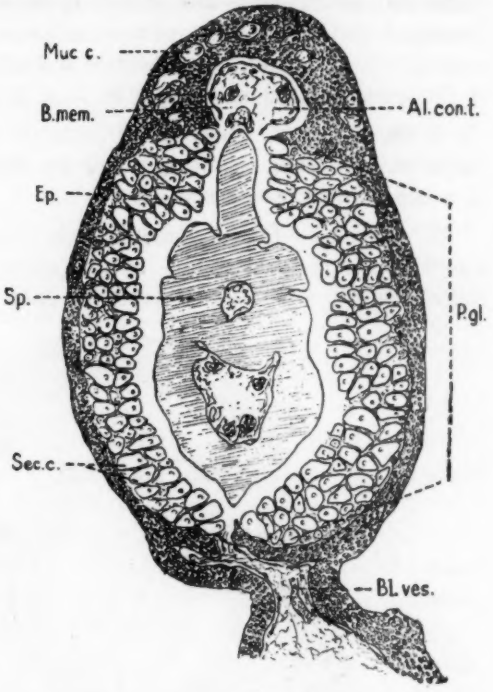

FIG. 2

FIG. 1. Vertical longitudinal section of the pectoral spine of *Heteropnuestes fossilis* (Bloch) showing the poison gland (Diagrammatic).

FIG. 2. Transverse section of the pectoral spine of *Heteropnuestes fossilis* (Bloch). (From an young specimen.)

cells of the epidermis, which are termed *rete mucosum* by Malpighi. They correspond to the pigmented capillary layer which gives rise to the secretory cells of the poison glands in *Trygon, Acanthias* and *Cistracion* (Evans, 1922). To begin with, these epithelial cells become distended with a secretory substance, and as they grow bigger in size they are pushed towards the spine by the newly distending secretory cells. Some of these cells are in an active state of secretion as can be judged from their appearance under high power of the microscope. The secretion constitutes the toxic substance of the gland. The nuclei of these cells become greatly enlarged and are feebly stained. These cells when fully distended liberate the poison by bursting. There are no blood vessels in the tissue which constitutes the

poison gland. Due to the distention of the epithelial cells and their considerable extension towards the spine, the alveolar connective tissue lying below the epidermis has disappeared in the region of the poison glands. It is noticed that the alveolar connective tissue with the basement membrane is therefore restricted to the posterior and to a smaller extent, anterior aspects of the spine. Blood vessels can be seen in the connective tissue.

If the poison is squeezed out from the spine on to a glass slide and examined in a fresh condition under the microscope it has the appearance of a pearly white substance.

2. *Plotosus.*—Fig. 3 represents the transverse section of the middle region of the pectoral spine. In this species also the poison gland, as in *Heteropnuestes*, is developed from the epidermis but in a slightly different

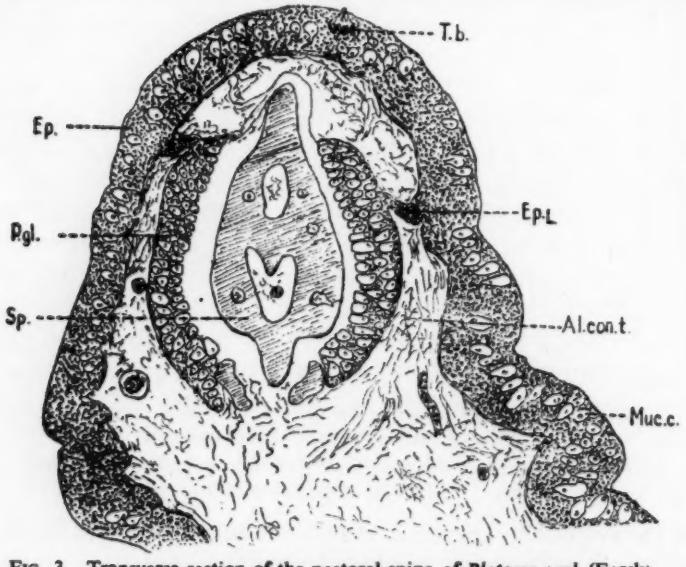

FIG. 3. Transverse section of the pectoral spine of *Plotosus arab* (Forsk).

manner. Unlike as in *Heteropnuestes*, the alveolar connective tissue with the basal membrane is continuous all round the spine. A thin layer of epidermis extends inwards in the form of a lamella, one on either side of the spine, which may be termed epidermal lamella. It is from this portion of the epidermis that the glandular cells of the poison glands appear to take their origin. The mode of formation of the secretory cells is the same as in *Heteropnuestes*. A separate sac and duct for the poison gland are absent in *Plotosus* as in the other species.

It may be pointed out here that the secretory cells of the poison glands are derived as has been described by Evans (1923) from the basal layers of the epidermis both in the Elasmabranchs—*Trygon*, *Acanthias* and *Cistracion* and presumably also in the two species of catfishes treated in this paper. But the mode of discharge of the poison in the two Siluroids is different from that in the Elasmabranchs. In the latter the poisonous secretory products are discharged outside directly whereas in the Siluroids they are pushed inwards and are eventually conveyed outside by a rupture of the skin at the tip of the spine.

Physiological Action of the Venom

Dr. P. M. N. Naidu, D.Sc., Veterinary Research Officer, has very kindly examined the effect of the poison of *Heteropnuestes* on the blood of horse and cow at the Serum Institute, Bangalore. Fresh poison was squeezed out of the spines of three specimens into a watch glass and dissolved in 3 c.c. of normal saline solution. Fresh blood was centrifuged and washed in saline solution. Two tubes of 1 c.c. each of the washed blood corpuscles of horse and another set of two tubes of the same quantity of corpuscles of the cow were taken. To one of the tubes of each set was added 5 drops of the venom in saline and the other two tubes were used as controls. They were incubated for half an hour at 37° C. After incubation it was noticed that in the tubes in which the poison was mixed, there was marked hæmolysis while there was no change in the control tubes.

Two frogs were inoculated each with 1 c.c. of the toxic fluid (venom in glycerine as stated under material and methods). It was observed that immediately after inoculation the frogs became dull and inactive. The lungs became distended. At intervals of two or three minutes the frogs exhibited tetanic convulsions, and collapsed in about 15 to 20 minutes after the inoculation. One specimen was immediately opened to study the condition of the heart. The heart had stopped working when the specimen was opened but two minutes later a faint pulsation was noticed for a half minute after which the beating stopped completely. Day has stated that the wounds from the pectoral spine of this fish causes tetanus occasionally among fishermen.

It is clear from the foregoing observations that the poison of *Heteropnuestes* has both neurotoxic and hæmolytic effect as it appears to affect both the nervous and blood systems.

The prick of the pectoral spine of *Heteropnuestes* is sometimes fatal to other fishes. Probably the mortality of other species of fishes in tanks

is due, to some extent, to this factor. To study the effect on another species of fish, six specimens of *Heteropnuestes fossilis* and six specimens of *Clarias batrachus* were let into a cement cistern. They were artificially fed and the water was changed periodically. After a few days, wounds with inflammation were noticed on the bodies of the *Clarias*. The fishes which were stung were dull and their skin and fins were eaten by *Heteropnuestes*. All the *Clarias* succumbed to these injuries except one which appeared to have become practically immune to the poison. The body of this specimen became greatly distorted with a bent back and its movements in the water were peculiar, almost grotesque and it died after living for six years.

In conclusion I wish to express my gratitude to Prof. A. Subba Rau, D.SC. (London), F.R.M.S., at whose suggestion this study was undertaken for his valuable criticisms and guidance and my best thanks to Mr. P. A. Ramakrishna Iyer, M.SC., for preparing sections of the pectoral spines and to Dr. P. M. N. Naidu, D.SC., for conducting the hæmolytic experiments.

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ABBREVIATIONS

Al. con. t.	.. Alveolar connective tissue.	*Muc. c.*	.. Mucous cell.
B. Mem.	.. Basement membrane.	*P. gl.*	.. Poison gland.
B. ves.	.. Blood vessel.	*Sec. c.*	.. Secretory cells.
Ep.	.. Epidermis.	*Sp.*	.. Spine.
Ep. L.	.. Epidermal lamella.	*T. B.*	.. Taste bud.

ON A SMALL COLLECTION OF PARASITIC NEMATODES FROM ANURA

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Received August 12, 1943

(Communicated by Dr. S. H. Lele, F.A.SC.)

The present paper describes two new species of Nematodes, one of which was parasitic in the common frog (*Rana tigrina*) and was collected by the writer. The second was obtained by Dr. G. D. Bhalerao from the Himalayan toad (*Bufo himalayanum*), and very kindly handed over to the writer for determination. The third species mentioned in this paper has already been described by the writer and the short note, here, records one or two additional characters not included in the original paper.

The writer takes this opportunity to express his best thanks to Dr. Bhalerao (Imperial Veterinary Research Institute, Izatnagar) for the material.

Family QUIMPERIIDÆ Baylis, 1930

Gendria ranarum n. sp.

The male measures 19·3 to 23·05 mm. in length and 0·4 to 0·45 mm. in maximum thickness, the female 26·1 to 31·6 mm. and 0·5 to 0·6 mm. respectively. The cuticle has very fine transverse striations and is inflated in the head region. Narrow, lobulated, lateral alæ are present in both sexes. In the male they begin at a distance of about 0·03 to 0·05 mm. and end at 0·52 to 0·80 mm. from the anterior extremity. In the female they begin at 0·08 mm. from the same end and apparently disappear in front of the excretory pore. The mouth is a shallow depression without any lips. Two pairs of submedian and a single pair of more laterally placed papillæ surround the mouth. A large dorsal tooth and two somewhat smaller subventral teeth project from the anterior end of the œsophagus, at the base of the mouth. The entire œsophagus is muscular and club-shaped posteriorly. At its anterior end it forms a comparatively large almost globular swelling. It is 0·45 to 0·66 mm. long in the male and 0·41 to 0·46 mm. in the female. The œsophagus opens into the intestine through a valvular apparatus. The nerve-ring encircles the œsophagus at 0·24 to 0·29 mm. in the male and

at 0·17 to 0·19 mm. in the female from the front end. The inconspicuous cervical papillæ are at 0·49 to 0·74 mm. in the male and at 0·44 to 0·52 mm. in the female from the anterior extremity; while the excretory

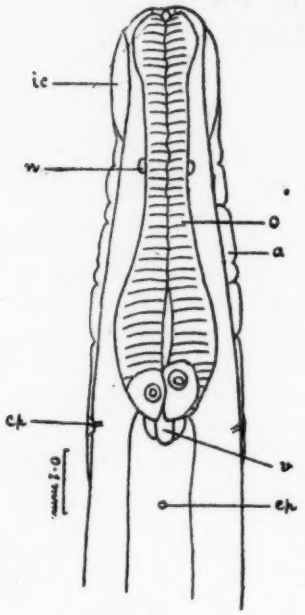

FIG. 1

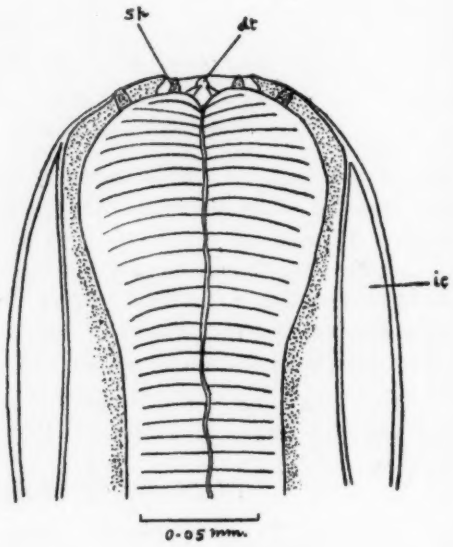

FIG. 2

FIG. 1. *Gendria ranarum.*—Anterior end of female, ventral view. *a.*, ala; *cp.*, cervical papilla; *ep.*, excretory pore; *ic.*, inflated cuticle; *n.*, nerve-ring; *O.*, œsophagus; *v.*, valves.

FIG. 2. *Gendria ranarum.*—Anterior end of female, ventral view. *ic.*, inflated cuticle; *dt.*, dorsal tooth; *sp.*, sub-ventral papilla.

pore is at a distance of 0·57 to 0·87 mm. in the male and at 0·53 to 0·63 mm. in the female from the same end.

The tail of the male, measuring 0·14 to 0·28 mm., is curved ventralwards and ends in a cuticular spine. Caudal alæ were not observed. There are nine pairs of rather large pre-anal and six pairs of smaller post-anal caudal papillæ and an unpaired median papilla situated in front of the cloacal opening in a line with the second posteriormost pre-anal pair. A single pair of small papillæ is situated on each of the two lips of the cloacal opening. A sucker-like organ is situated at a distance of 0·81 to 1·38 mm. from the posterior end. The two equal and sickle-shaped spicules are 0·33 to 0·57 mm. long. A small chitinised accessory piece is present.

The tail of the female mesures 0·155 to 0·195 mm. and terminates in a sharp cuticular spine. A pair of caudal papillæ is situated at 0·07 to 0·09 mm.

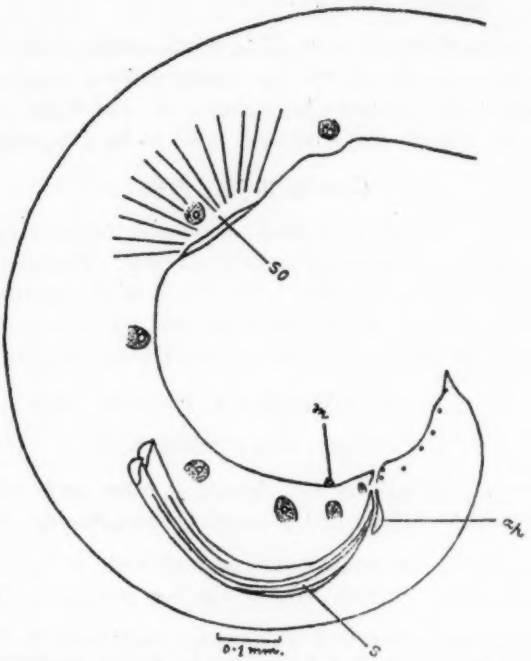

FIG. 3. *Gendria ranarum*.—Caudal end of male, lateral view. *ap.*, accessory piece; *m.*, median papilla; *S.*, spicule; *So.*, sucker-like organ.

from the tip of the tail. The vulva is at a distance of 11·7 to 13·3 mm. from the posterior end. The muscular vagina runs anteriorwards for a distance of 0·43 mm. and gives off two opposed uterine branches, which are muscular for some length. The eggs measure 0·045 to 0·065 mm. × 0·04 to 0·053 mm. and their contents are segmented.

Upto present two species of the genus *Gendria* Baylis 1930, have been described. The first, *G. tilapiæ* is the genotype and the second, *G. brevispiculum* was described by the writer in 1941. The species described above differs in many respects from these two. But the striking characteristic which distinguishes it from the abovementioned two forms is the presence of lateral cervical alæ. This character, along with others, justifies the creation of a new species to accommodate the worm described here and accordingly it is named *Gendria ranarum*.

Habitat.—Small intestine.

Host.—Rana tigrina.

Locality.—Talegaon (Poona).

In the generic diagnosis of *Gendria*, Baylis (1930) mentions among other characters, " Cervical alæ absent, but cuticle inflated anteriorly to form an oval expansion." In view of the presence of the lateral cervical alæ in *G. ranarum*, the generic diagnosis will have to be emended a little.

Gendria Baylis, 1930

Quimperiidæ. Mouth a shallow depression without definite lips, but with a dorsal and two subventral teeth at its base. Oesophagus not divided into two portions, but somewhat club-shaped both anteriorly and posteriorly. Cervical alæ may or may not be present, but cuticle inflated anteriorly to form an oval expansion. An accessory piece present in the male.

Family OXYURIDÆ Cobbold, 1864

Cosmocercoides bufonis n. sp.

The worms, collected by Dr. Bhalerao from *Bufo himalayanum*, on examining, proved to belong to the genus Cosmocercoides Wilkie, 1930.

The parasites are relatively stout and both ends of the male and female are curved ventrally. Narrow lateral alæ are present in both sexes.

The male measures 4·6 to 4·92 mm. in length and 0·35 to 0·425 mm. in maximum thickness, the female 5·15 to 6·3 mm. and 0·45 to 0·55 mm. respectively. The transverse striations on the cuticle are 0·0025 mm. apart. The head is not marked off from the body. The mouth is surrounded by three equal lips, one dorsal and two subventral. Each lip is produced forward into a cuticular lobe and bears externally a single pair of papillæ placed at the anterior corners.

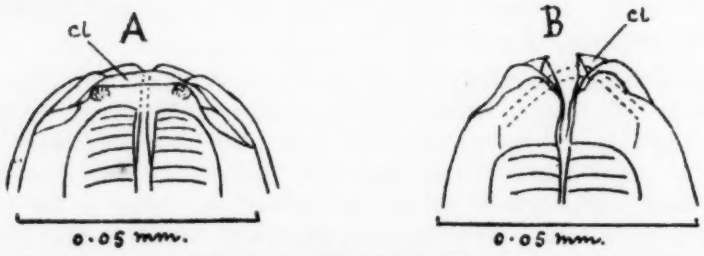

FIG. 4. *Cosmocercoides bufonis.*—A. Anterior end of female; dorsal view. B. Anterior end of female; ventral view. *cl.*, cuticular lobe of the lip.

The lateral alæ begin from the head and run posteriorwards. They are extremely narrow in the beginning but at a distance of about 0·17 to 0·175 mm. in the male and 0·2 to 0·23 mm. in the female from the anterior end, become relatively broader. In the male these lateral alæ continue backwards and apparently end a little in front of the cloacal opening, while in the female they extend beyond the anal opening and terminate at 0·15 to 0·19 mm. from the posterior end; but in some females they appear to continue almost to the posterior end.

The buccal cavity is about 0·15 to 0·175 mm. and is followed by a pharynx, measuring 0·06 to 0·07 mm. in the male and 0·07 to 0·075 mm. in the female. The œsophagus is divided into two parts, a long cylindrical portion and a bulb. The œsophageal bulb measures (excluding the neck) 0·125 to 0·135 mm. × 0·14 to 0·15 mm. in the male and 0·15 to 0·16 mm. × 0·165 to 0·185 mm. in the female. There is a small prebulbar swelling separated from the bulb by a short neck. The entire œsophagus is 0·58 to 0·66 mm. long in the male and 0·85 to 1·06 mm. in the female. The nerve ring is at a distance of 0·24 to 0·26 mm. in the male and at 0·36 to 0·38 mm. in the female from the anterior end. The excretory pore is situated at 0·36 to 0·37 mm. in the male and at 0·5 to 0·68 mm. in the female from the same end. The cervical papillæ are either at the level of the nerve-ring or a little behind it.

The tail of the male is 0·2 to 0·24 mm. and tapers to a fine point. Caudal alæ were not observed. Caudal papillæ are numerous and of two kinds. Some are simple while others are what Wilkie (1930) describes, "large papillæ having a surrounding ring of tubercles, purely cuticular in structure, and unsupported by any internal skeleton." There are 9 to 13 pre-anal pairs of these complex papillæ, subventral in position. Besides these, simple papillæ, varying in number, are present in the pre-anal region. These are irregularly arranged and may or may not be present in pairs. There is a single para-cloacal pair of complex papillæ. The post-anal region appears almost studded with papillæ. Their number and situations vary in individual specimens but amongst these are found pairs which are constant and can, therefore, be relied upon for specific diagnosis. Only three of the postanal pairs are of the complex type, the remaining being simple. Two of these pairs are subventral and one lateral. Of the constant simple post-anal pairs (counting from the posterior end), Nos. 1, 2, 4 and 6 are ventral; Nos. 3 and 8 dorsal. No. 5 is either dorsal or subdorsal but in some specimens it is lateral. No. 7 is subdorsal. Nos. 9 and 10 are subventral. The lips of the cloacal opening bear a single median papilla each. The two

spicules measure 0·19 to 0·26 mm. in length. There is a chitinised accessory piece 0·12 to 0·145 mm. long.

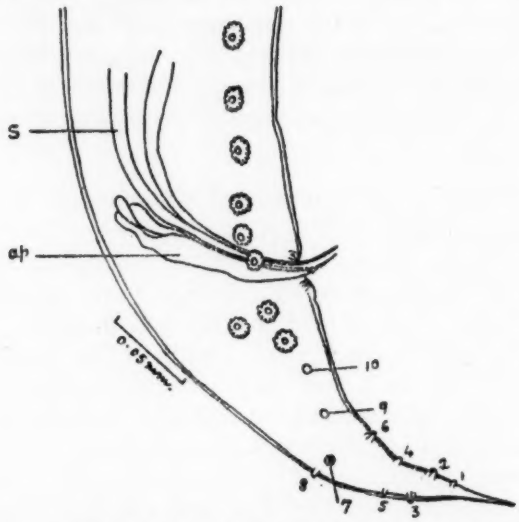

FIG. 5. *Cosmocercoides bufonis*.—Posterior end of male to show post-anal papillæ; lateral view. *ap.*, accessory piece; *S.*, spicule; 1 to 10, post-anal pairs of simple caudal papillæ (only 5 pre-anal pairs of the complex papillæ are included in the figure).

The tail of the female is 0·23 to 0·26 mm. long and terminates in a sharp point. It bears in the majority of specimens, two pairs of caudal papillæ, the posterior pair at 0·065 to 0·075 mm. and the anterior at 0·09 to 0·155 mm. from the posterior extremity. The vulva is situated at a distance of 2·20 to 2·7 mm. from the posterior end. The muscular vagina is about 0·55 mm. long and runs anteriorwards parallel to the body wall to a distance of about 0·24 mm. from the vulva. It then turns back and gives rise to the two large opposed uterine branches either at the level of the vulva or just posterior to it. The eggs measure 0·075 to 0·08 mm. × 0·04 to 0·045 mm. and contain a larva.

Of the species assigned to the genus *Cosmocercoides*, the present form very closely resembles *C. variabilis* (Harwood, 1930). However, it can be distinguished from the latter in the following respects. The lateral alæ begin from the head and not from the middle of the œsophageal region as in *C. variabilis*. All the three lips are equal, conspicuous and produced forward into a cuticular lobe, while the lips in *C. variabilis* are small and inconspicuous the dorsal being large. There are in all 13 to 16 pairs of complex papillæ, the number

for *C. variabilis* being 15 to 20. While there appear to be no simple pre-anal papillæ in *C. variabilis*, as many as 7 pairs of these have been observed in the present form. There are at least 10 pairs of simple post-anal papillæ constantly present in the species here described while in *C. variabilis* there are only 4, all others being variable. The spicules of the present form are relatively smaller than those in Harwood's species.

These differences are sufficient to show that the worms described above are distinct from *C. variabilis* and therefore, it is proposed to refer them to a new species *Cosmocercoides bufonis*.

Habitat.—Cæcum.

Host.—Bufo himalayanum.

Locality.—Muktesar (Himalayan foot-hills).

Family CAMALLANIDÆ Railliet and Henry, 1915

Camallanus baylisi Karve, 1930

A number of worms belonging to this species were obtained from the small intestine of *Rana tigrina*, the locality of the host being Talegaon near Poona. The present material agrees, in all essential respects that described in the original paper, except that the females are somewhat larger. The largest female is 22·9 mm. long and 0·55 mm. in maximum breadth. No mention could be made, in the original paper, as regards the caudal papillæ in the female as they were not observed. The females from the present batch of worms show distinctly a single pair of caudal papillæ.

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STUDIES IN GRAMS OF C. P. & BERAR

1. Biology of Gram Flower

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Introduction

Gram (*Cicer arietinum* L.) belongs to the family Leguminosæ and subfamily Papilionatæ. Among the pulses of the Province this crop occupies by far the largest area and has got a variety of uses in the dietary of man. In order to improve the yielding capacity of this crop, selection and hybridisation work was undertaken in this Department. The study of the biology of flowers forms the preliminary step in the breeding work of any crop. This type of work has been done at various centres by different workers in India; but as the climate and soil of this Province differ from those of other places, it was thought very essential to make a separate study of the biology of flower which is the subject-matter of this paper.

Previous Literature

Howard[1] (1915) observed the opening of the flowers in gram at Pusa. He found them to open at 9 A.M. and close the same day before sunset. They also open the next day between 8-30 A.M. and 11 A.M. and close finally in the afternoon. The length of time the flowers remained open varied from 7 to 15 hours. The lower flowers opened first. The anthers dehisce in the bud stage thus ensuring self-pollination.

Ayyar and Bal Subramanyan[2] (1935), however, found that most of the flowers that opened on the first day, as a rule, did not open on the second day. The second opening was earlier in the day than the first one. Dehiscence and pollination occurred a day before the opening of the flower.

Kadam, Kulkarni and Patel[3] (1938) observed that the opening of flowers takes place between 11 A.M. to 2 P.M. The second opening on the following day is earlier than the first. They also observed some flowers opening for the third time. Flowers close soon after sunset.

Description

The inflorescence and the floral mechanism.—The flowers are borne singly on the branches opposite to the leaves on the short peduncle which are jointed. There was, however, no type with pedicel possessing double flowers like Type No. 83 described by Shaw and A. R. Khan[4] (1931) in their classification. It is found that the lowest buds open first of all. In the study of the flowers, it was observed that in the previous evening before their opening the stamens are far below the level of the stigma, arranged in a circle; but before the opening of the flower next day, they come up to the level of the stigma and when the flower is open the stigma is found to be completely covered with the pollen grains. The flowers generally remain open the whole day and close down in the evening. On the second day they have been necessarily found to open again and finally close, after which the corolla sheds down. This is in contradiction of the findings of Bal Subramanyan and Iyer[2] (1935) who state that the flowers, as a rule, have no second opening. In the case of pink flowers the corolla first assumes light blue colour which goes on fading gradually, ultimately turning brown at the time of shedding. In the case of white corolla, it begins to turn brown and sheds. Kadam, Kulkarni and Patel[3] (1938) have observed that in many cases the flowers open for the third time on the third consecutive day. No such case was, however, observed in the present studies.

Material and Methods

(A) 25 Buds which were likely to start opening on the next morning were selected and labelled in the evening. The corolla of these buds was removed very gently early in the next morning and observations regarding the time when the dehiscence of the anthers started and also the time when it was completed were recorded in each case. The observations were made at an interval of fifteen minutes and were continued till 12 A.M. One set of 25 buds was observed on a clear day and the other one was recorded on the cloudy day just to see whether there was any difference due to temperature.

(B) The observations were recorded for opening of the flowers on both the days (clear and cloudy days), as far as possible, in all the cultures. In

each culture 25 buds were selected and labelled a day previous to the expected opening and on the following day, observations were made. On the 15th December 1933, 25 buds each from four cultures, *viz.*, Nos. 28/10; 28/11; 12 and G-11 were selected and observations, as to the opening of the flowers, were recorded on the 16th December 1933 as detailed below. A similar set of observations was made on the 14th January 1934, in the following six cultures, *viz.*, Nos. 24/3; 25/12; 37/4; 38/1; 39/4 and G-11. These observations are recorded in Tables I–VI, attached herewith in Appendix. On the first day of the observations three buds were damaged and, therefore, the total number of buds observed is 247. On the second day a large number of buds was found to have been damaged by the insect attack (*Heliothes obsol ta*), and only 199 buds were left for observation.

A detailed study of the dehiscence of the anthers and the opening of flower was made during the months of December and January in the year 1933–34 at the Agricultural Research Institute, Nagpur, under Mr. D. N. Mahta, B.A. (Oxon.), the then Second Economic Botanist, C.P., Nagpur. Observations were made as under:—

(A) The dehiscence of the anthers.

(B) Opening of the flowers:—

(1) *Appearance of the opening.*—In this, the time when opening of the standard started has been recorded in case of each bud.

(2) *Time taken by the flower to open completely.*—This is the time when the flower starts opening till it is fully open. This has been recorded in minutes for each bud.

(3) *Time taken by the flower to close completely.*—In this the time taken by the flower from the time the closing started till it closes completely has been recorded.

(4) *Time of closing.*—Here the time, when closings of the standard started, has been recorded.

(5) *Time during which the flowers remain completely open.*—Here the time, in hours and minutes, has been calculated from the time the flower is completely opened to the time when closing starts.

(6) *Time from the opening of the flower* starts till it completely closes has been calculated.

Results

So far as bursting of anthers is concerned, it was found that it takes place between 8–45 to 10–30 A.M. on the clear day. On cloudy day it was started as early as 8-15 A.M. and continued till 9 A.M.

Observations on the opening of flowers are tabulated below:—

Data of the results for opening of the flowers

Particulars	First opening	Second opening	Table Nos.
I. Time of opening—			
(*a*) Range	8 A.M.–2-30 P.M.	8 A.M.–10 A.M.	I
II. Time taken to open—			
(*a*) Range	30–240 mins.	30–180 mins.	II
(*b*) Mean	101·5	86·9	
(*c*) Coeff. of var.	14·15	14·4	
(*d*) Probable error	±1·50	±1·37	
III. Time taken to close—			
(*a*) Range	15–180 mins.	15–180 mins.	III
(*b*) Mean	55·4	64·2	
(*c*) Coeff. of var.	7·45	13·7	
(*d*) Probable error	±0·79	±1·93	
IV. Time of closing—			
(*a*) Range	4 P.M.–6-30 P.M.	3-30 P.M.–6-30 P.M.	IV
V. Duration till the flower remains open—			
(*a*) Range	1½–7½ hrs.	3–9 hrs.	V
(*b*) Mean	5·70	6·62	
(*c*) Coeff. of vari.	0·037	0·69	
(*d*) Probable error	±0·04	±0·050	
VI. Total duration of opening (from the time the opening started to the time when closing completed on two consecutive days)—			
(*a*) Range		11–20½ hrs.	
(*b*) Mean		15·51	
(*c*) Coeff. of var.		0·74	
(*d*) Probable error		±0·0105	

Duration of opening of flowers in December and January

Particulars	December 1933		January 1934		
	First opening	Second opening	First opening	Second opening	
I. Duration (from the time when the flower opened completely till the time when closing started)—					
(*a*) Range ..	3–6¼ hrs.	3–8½ hrs.	4½–9 hrs.	1½–7½ hrs.	
(*b*) Mean ..	5·19	6·09	6·82	5·22	
(*c*) Coeff. of var. ..	2·00	7·6	0·54	0·69	
(*d*) Probable error	±0·13	±0·312	±0·526	±0·561	
II. Duration (from the time when opening started till the flower closed completely)—					
(*a*) Range ..	6–10 hrs.	6–9½ hrs.	3½–9½ hrs.	6½–9½ hrs.	VI
(*b*) Mean ..	7·8	8·8	6·8	8·2	
(*c*) Coeff. of var. ..	0·98	0·03	0·70	0·50	
(*d*) Probable error	±0·66	±0·02	±0·058	±0·0404	

Discussion of the Results

(A) So far as the bursting of anthers is concerned, it has been observed that it takes place between 8-45 A.M. to 10-30 A.M. on the clear day depending upon the developmental stage of the particular bud. Observations were also made on a cloudy day, when the bursting of the anther-sac was found to have started as early as 8-15 A.M. So also in all of the flowers under observation on the day the dehiscence began from 8-15 to 9 A.M. The probable reason for this early dehiscence is the possible rise in the temperature on such a day.

(B) In the present investigation, flowers have been observed to open as early as 8 A.M. and continue till 2-30 P.M. No flower was observed to have opened after that time. This is much earlier than the opening of flower observed by Kadam, Kulkarni and Patel[3] at Kundewadi, where it takes place between 11 to 2 P.M. At Pusa, however, the opening takes place between 9 to 4 P.M. as mentioned by Howard[1] (1915). Data recorded in Table I show that the flowers opened an hour earlier on the 16th December 1933, than on 14th January 1934. It is difficult to assign this to any particular cause but it may be due to difference in some environmental factor, possibly temperature on the particular day. As has already been said, the opening begins at 8 A.M. and continues till 2-30 P.M. (Table I). It is clear that the frequencies reach a peak point a little after 10 A.M. Also that the maximum number of flowers open between 10 and 11 A.M., the number falling steeply again after that time. There are only two flowers that open after 1 P.M.

As regards the time taken for the opening of the flowers, it varies from 30 minutes to 240 minutes; but a considerable majority of the total flowers observed was found to have taken from 1 hour to two and a quarter hours to complete the opening. The mean time taken for complete opening is calculated to be 101·5 minutes with a probable error of $\pm$ 1·5.

So far as the second opening of the flowers on the following day is concerned, it has been observed that the time range is comparatively smaller than the first opening on the previous day. All the flowers opened between 8 A.M. and 9-30 A.M. on the second day. The time range is comparatively smaller than the finding of Howard[1] (1915) at Pusa. Secondly in all the cases the flowers not only started opening earlier than on the previous day but also took less time to open completely. The mean time taken for the opening is 86·9 with a probable error of $\pm$ 1·37.

The flowers begin to close on the same day from 4 P.M. onwards and continue till 6-30 P.M. The time of closing and their frequencies are tabulated in Table IV. It is obvious that most of the flowers close between

5 and 6 P.M. The closing time thus fairly coincides with the findings of Howard, Howard and A. R. Khan[1] (1915) and Kadam, Kulkarni and Patel[3] (1938). It has also been observed that the time taken by the flower to close completely varies from 15 minutes to 180 minutes, though 95% of the observed flowers have taken between 30–90 minutes for closing. The average time taken for closing has been calculated to be 55·4 minutes with a probable error of ± 0·79.

The time at which the flowers mostly start closing on the second day ranges between 4 and 6 P.M. though it has been observed that one flower began closing as early as 4–30 P.M., while two others started closing so late as 6-30 and 7-30 P.M. respectively. Thus the time of second closing unlike the time of opening closely coincides with the time of closing on the first day.

It appears from the data that opening of flowers started earlier in December 1933 (for both openings) than in January 1934. The result is that the total duration of opening—combined duration of first and second opening on the two consecutive days in December 1933—has been found to be more than that in January 1934. So also it has already been shown that the second opening of flowers, both in December and January, has been found to take place earlier in the day than the first opening. The ultimate result of this is that the total duration of the first opening is less than on the second day. It has been calculated that the average total duration of opening from the time it begins opening till it closes completely both on the first and second day is nearly 15½ hours (15·51) with a probable error of ± 0·0105.

Summary and Conclusion

A detailed study of the dehiscence of anthers and the opening of the flower was made during the months of December and January 1933–34. Observations given in brief below were made in ten cultures selected for this purpose.

(1) The bursting of anthers takes place between 8-45 A.M. and 10-30 A.M. on a clear day. On cloudy day bursting starts as early as 8–15 A.M. the probable cause for the early bursting is the rise in the temperature.

(2) In gram, the flowers generally remain open the whole day and close in the evening. On the second day they again open and close finally after which the corolla sheds down. No case of opening on the third day was observed as reported by Kadam, Kulkarni and Patel[3] (1938). All the flowers without an exception opened on the second day.

(3) Flowers begin opening from 8 A.M. and continue till 2–30 P.M., the maximum number opening between 10 to 11 A.M. This is much earlier

than the opening observed at Kundevadi by Kadam, Kulkarni and Patel[3] (1938) and Howard[1] (1915) respectively.

(4) In December the flowers open an hour earlier than in January. This may be due to some environmental factor, possibly temperature on the particular day. In January the flowers open between 10 and 10–30 A.M.

(5) The time taken by the flowers to open completely varies from 30 to 240 minutes. The mean time taken for complete opening is calculated to be 101·5 minutes with a P.E. of ± 1·5.

(6) So far as the opening on the second day is concerned both in December and January, the time range is comparatively smaller than the opening on the previous day. The flowers opened between 8 to 9–30 A.M. They have also taken less time to open completely. The mean time taken as calculated is 86·1 with a P.E. of ± 1·37 minutes.

(7) The closing of the flowers on the first day takes place between 4 to 6-30 P.M. This observation is in accord with the findings of Howard, Howard and Khan[1] (1915) and Kadam, Kulkarni and Patel[3] (1938). The time taken to close the flower varies from 15 to 180 minutes though 95% were observed to have taken 30–48 minutes. The average time taken has been calculated to be 55·4 with a P.E. of ± 1·079. The time of closing on the second day fairly coincides with the time of closing on the first day.

(8) The combined duration of opening on both the days in December has been found to be more than the combined duration of opening in January. This is due to the fact that the opening in December started earlier than in January.

Duration of opening of the flower is as follows:—

	December	*January*
1st	7·8 ± 0·066	6·8 ± 0·058
2nd	8·8 ± 0·02	8·2 ± 0·040

The average combined duration of flowers is 15·51 with a P.E. of ± 1·00105.

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Appendix

TABLE I

Time of opening of flowers

Time	First opening											Second opening										
	16–12–33 to 14–1–34											17–12–33 to 15–1–34										
	Culture Numbers and Frequencies																					
	*1	2	3	4	5	6	7	8	9	10	Total	1	2	3	4	5	6	7	8	9	10	Total
7–30— 8 A.M.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	1
8 — 8–30 ,, ...	..	..	..	..	..	..	..	..	..	..	..	22	19	8	4	14	5	21	23	25	10	151
8–30— 9 ,, ...	1	4	6	6	..	..	6	2	1	..	26	..	..	..	..	9	11	2	2	..	7	31
9 — 9–30 ,, ...	..	..	..	..	..	1	..	..	1	..	2	..	..	..	..	3	6	..	..	..	5	14
9–30—10 ,, ...	10	2	6	7	9	4	7	7	17	7	76	1	1	..	..	..	..	..	..	..	..	2
10 —11 ,, ...	12	15	10	12	14	16	10	12	5	9	115	..	..	..	..	..	..	..	..	..	..	..
11 —12 Noon ..	2	3	..	..	2	3	1	3	..	6	19	..	..	..	..	..	..	..	..	..	..	..
12 — 1 P.M.	..	..	..	..	2	2	..	..	1	2	7	..	..	..	..	..	..	..	..	..	..	..
1 — 2–30 ,, ...	..	..	..	..	..	..	..	1	..	1	2	..	..	..	..	..	..	..	..	..	..	..
TOTAL ..	25	24	22	25	27	25	24	25	25	25	247	23	20	8	4	26	23	23	25	25	22	199

TABLE II

Time taken by the flower to open

(*From the time opening started to the time wnen it is completely opened*)

Minutes	First opening											Second opening									
	16–12–33							14–1–34				17–12–33							15–1–34		
	Culture Numbers and Frequencies																				
	1	2	3	4	5	6	7	8	9	10	Total	1	2	3	4	5	6	7	8	9	10	Total
30- 60	10	13	7	7	2	6	7	4	12	6	74	8	18	1	1	3	2	9	4	4	1	51
60– 90	..	..	..	..	12	6	9	10	10	10	57	..	..	..	..	4	15	9	16	18	9	71
90–120	12	5	9	12	11	9	8	11	3	1	81	15	1	7	3	16	3	5	5	3	8	66
120–150	..	..	..	..	2	3	..	..	..	7	12	..	..	..	..	3	2	..	..	..	4	9
150–180	3	3	5	4	..	..	..	..	..	1	17	..	1	..	..	..	1	..	..	..	..	2
180–240	..	3	1	2	..	..	..	..	..	..	6	..	..	..	..	..	..	..	..	..	..	..
TOTAL ..	25	24	22	25	27	25	24	25	25	25	247	23	20	8	4	26	23	23	25	25	22	199
	Mean 101·5 Coeff. var. 14–15 P.E. 1·5											Mean 86·9 Coeff. var. 14·4 P.E. 1·37										

TABLE III

Time taken by the flower to close

(From the time closing starts till it is completely closed)

Minutes	First opening											Second opening										
	16–12–33										14–1–34	17–12–33										15–1–34
	Culture Numbers and Frequencies																					
	1	2	3	4	5	6	7	8	9	10	Total	1	2	3	4	5	6	7	8	9	10	Total
1– 30	..	..	6	13	4	2	..	1	..	9	35	..	..	..	..	10	10	2	2	4	10	38
30– 60	14	8	14	9	20	20	23	16	15	14	153	14	18	6	3	9	8	3	7	8	12	88
60– 90	11	16	..	..	3	3	1	8	10	2	55	..	..	1	..	4	5	18	16	13	..	57
90–120	..	..	2	1	..	..	..	..	..	..	3	7	2	1	1	3	..	..	..	..	..	14
120–150	..	..	..	1	..	..	..	..	..	..	1	2	..	..	..	..	..	..	..	..	..	2
TOTAL ..	25	24	22	25	27	25	23	25	25	25	247	23	20	8	4	26	23	23	25	25	22	199
	Mean 55·4 Coeff. var. 7·45 P.E. 0·79											Mean 64·3 Coeff. var. 13·7 P.E. 1·93										

* Culture numbers 1–10 refer to original numbers 28/10 to G–11 mentioned on page 80.

TABLE IV

Time of closing the flowers

Time	First opening											Second opening										
	16-12-33 to 14-1-34											17-12-33 to 15-1-34										
	Culture Numbers and Frequencies																					
	1	2	3	4	5	6	7	8	9	10	Total	1	2	3	4	5	6	7	8	9	10	Total
4—4-30 P.M.	..	..	..	..	1	2	..	..	..	2	5	..	..	..	..	..	1	..	..	..	..	1
4-30—5 ,, ...	..	..	..	..	15	21	..	8	6	11	61	5	6	5	..	5	17	4	9	5	14	70
5 —5-30 ,, ...	..	..	..	..	5	..	24	16	19	12	76	13	10	2	1	15	4	8	14	16	8	101
5-30—6 ,, ...	14	7	18	16	3	2	..	..	..	..	60	5	4	1	2	6	1	1	2	4	..	26
6 —6-30 ,, ...	11	17	4	9	3	..	..	1	..	..	45	..	..	..	1	..	..	..	..	..	..	1
TOTAL ..	25	24	22	25	27	25	24	25	25	25	247	23	20	8	4	26	23	23	25	25	22	199

TABLE V

Duration of opening of flowers

(From the time the flower is completely open till it starts closing

Hours	First opening											Second opening										
	16–12–33							14–1–34				17–12–33							15–1–34			
	Culture Numbers and Frequencies																					
	1	2	3	4	5	6	7	8	9	10	Total	1	2	3	4	5	6	7	8	9	10	Total
1 –3	..	..	..	..	1	..	..	1	..	..	2	4	..	..	..	..	..	..	..	..	..	4
3 –3½	..	..	..	1	1	6	..	1	..	..	9	..	2	..	..	..	2	..	..	..	..	4
3½–4½	..	..	5	6	15	13	..	5	2	2	48	..	..	..	..	..	..	..	..	..	..	..
4½–5½	25	24	16	18	4	4	24	14	5	23	157	7	1	6	1	1	4	..	..	..	2	22
5½–6½	..	..	1	..	6	1	..	3	18	..	29	2	4	1	2	9	15	2	8	5	16	64
6½–7½	..	..	..	..	..	1	..	1	..	..	2	9	9	..	1	14	1	14	13	16	4	81
7½–8½	..	..	..	..	..	..	..	..	..	..	..	1	4	1	..	2	1	7	4	4	..	24
TOTAL ..	25	24	22	25	27	25	24	25	25	25	247	23	20	8	4	26	23	23	25	25	22	199
	Mean 5 hours Coeff. var. 0·037 P.E. 0·04											Mean 6·62 Coeff. var. 0·69 P.E. 0·05										

TABLE VI

Duration of opening

(From the time the opening started to the time when closing completed)

Hours	December 1933									January 1934										
	First opening				Second opening				Total	First opening					Second opening					Total
	Culture Numbers and Frequencies																			
	1	2	3	4	1	2	3	4		5	6	7	8	9	10	5	6	7	8	9	10	
3–4	..	..	..	..	..	..	..	..	..	1	..	..	1	..	3	..	..	..	..	..	..	5
4–5	..	..	..	..	..	..	..	..	..	2	2	..	1	..	1	..	..	..	..	..	..	6
5–6	..	..	..	..	..	..	..	..	..	1	7	1	4	2	8	..	..	..	..	..	..	23
6–7	..	10	9	8	1	1	..	..	38	14	14	8	11	9	3	..	5	..	..	..	..	66
7–8	..	9	9	5	7	5	5	..	45	6	1	9	6	12	10	..	14	6	8	..	3	89
8–9	..	5	2	5	7	12	10	2	47	3	..	6	1	2	..	3	17	16	15	16	9	90
9–10	..	1	4	3	3	5	4	..	20	..	..	..	1	1	..	17	6	1	2	4	11	16
10–10½	..	..	..	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..
TOTAL	..	25	24	22	25	23	20	8	4	151	27	24	24	25	26	25	26	23	23	25	25	22	295
Mean	..	..	..	..	..	7·8				8·8			6·8			8·2						
Coeff. var.	..	..	..	..	..	0·98				0·03			0·7			0·5						
Probable error	..	..	..	..	..	0·66				0·02			0·058			0·0404						

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ADAPTATION OR ACCOMMODATION IN UNSTRIATED MUSCLE

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Irritable tissues are known to adapt or accommodate themselves to the continued presence of a stimulus by becoming less responsive. This adaptation depends upon the particular kind of tissue, and upon the nature of the stimulus. In unstriated muscle, adaptation is very conspicuous, but it depends upon the nature of stimulation (Singh, 1936; 1937; 1938*a*, *b*, *c*, *d*, *e*, *f*; 1939*a*, *b*; 1943*a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*; Rao and Singh, 1940; Singh and Mrs. Singh, 1943; Gokhale and Singh). It adapts rapidly to alternating current, but very slowly to some chemical substances. Not only it adapts to excitation (Singh, 1938*c*) but also to inhibition (Singh, 1942*b*), the tendency for the muscle being to produce an opposite change. In this paper various factors which influence adaptation in unstriated muscle are described.

Results

When *Mytilus* muscle is stimulated with alternating current (A.C.), the tension may subside while the current is passing through the muscle. Two classes occur; in potassium sensitive muscles, when the tension has declined, the sensitivity to potassium increases or at first decreases and then increases, while in comparatively insensitive muscles, the sensitivity decreases or shows no increase. The same effects are noted in frog muscle; here the sensitivity at first decreases and then increases.

On cessation of the current also, two phenomena are noted; the sensitivity to potassium either increases or decreases or shows no increase. When *Mytilus* muscle is stimulated intermittently (A.C. 8 V/10 sec. per min.), fatigue occurs. Again two phenomena are noted, either increase or decrease of sensitivity. This is beautifully shown by the A.C. off-contracture (Figs. 1, 2). It increases with fatigue in potassium sensitive muscle and decreases in comparatively insensitive muscle, if it occurs in the latter.

Reactions of potassium insensitive Mytilus muscles.—When these muscles are stimulated with A.C. the tension subsides to zero. This adaptation appears to be analogous to the "accommodation" in nerve described by Hill (1936). The tension subsides when " U " rises higher than " V " (Singh, 1943 *d*).

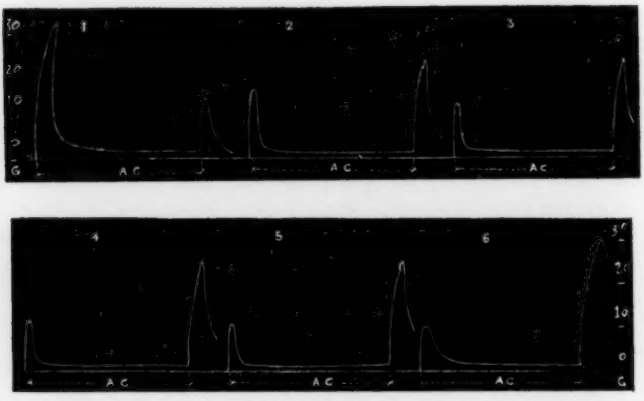

FIG. 1. *Mytilus* muscle. Stimulation with A.C. 10 V/5 min. per 10 min.

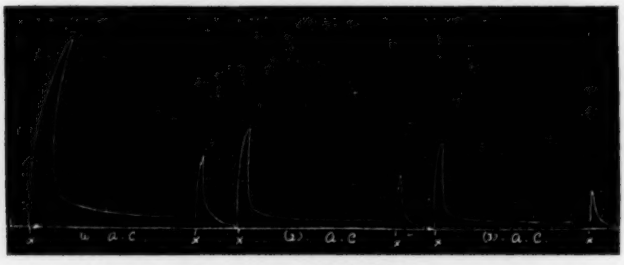

FIG. 2. Same as in Fig. 1

The above view is supported by the fact that calcium increases adaptation (Singh, 1938*d*). Further when the current is stopped, the sensitivity to A.C. must return to its original value. Calcium retards this recovery (6 experiments; Fig. 3). When the muscle is stimulated with A.C. intermittently calcium increases fatigue (6 experiments; Fig. 4). Such summer *Mytilus* muscles, when placed in *Mytilus* saline lose weight (Singh, 1938 *a*, *d*), behaving as if they contain excess of calcium; in calcium the muscle loses weight.

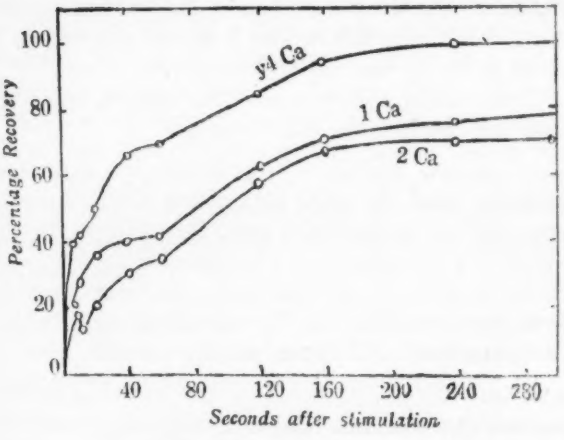

FIG. 3

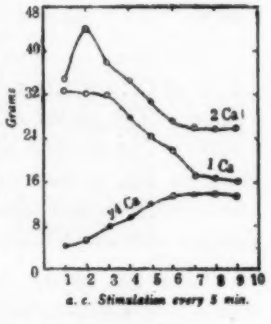

FIG. 4

FIG. 3. *Mytilus* muscle. Effect of calcium on recovery of excitability in potassium insensitive muscle after stimulation with A.C. 10 V/5 min.

FIG. 4. *Mytilus* muscle. Effect of calcium on fatigue to A.C. 10 V/10 sec. in potassium insensitive muscle.

The above adaptation appears to be brought about by liberation of calcium. Liberation of calcium by muscular activity has been shown by Zagami (1928), Wacker (1929); Dill, Talbot and Edwards (1930); Beznak (1931); Goldberg *et al.* (1931); Cloetta, *et al.* (1934); Weise, (1934); Coombs *et al.* (1934): Miko and Pala (1927): Bachromojew and Pawlowa (1935).

In *Mytilus* muscle excess of magnesium increases the latent period of the A.C. contraction. In some muscles, in 0·1 M $MgCl_2$, current was passed for 10 sec. and latent period was about 4–5 sec. after which the muscle contracted abruptly (6 experiments). Potassium (0·01–0·02 M KCl) similarly increased the latent period of the contraction produced by stretch and release (6 experiments) and that of the barium contraction (3 experiments) potassium and magnesium increase accommodation in nerve (Solandt, 1936), so that the latent period is probably the time required by "V" to catch "U".

When A.C. is passed for 5 min. the muscle may remain contracted during the period (adaptation is slow). If contracture is induced by the exclusion of calcium from the saline, the current merely causes relaxation; the rise of "V" in the former case produced contraction, and in the latter case, relaxation. On cessation of the current, the contracture recurs abruptly; this shows that the rise of "V" practically lasts during the passage of the current only. If the muscle is stimulated for a short duration (A.C. 10 V/10

sec.) then, when the stimulus is over, fatigue persists to A.C. and potassium and tone is neutralised for 3–4 sec. As this fatigue is due to adaptation, it suggests that " U " returns to its normal value more slowly than " V ", because the rise of " V " lasts during the passage of the current, while the rise of " U " persists after the cessation of the current.

Reactions of potassium sensitive muscles.—Two factors are antagonistic to A.C.; one is adaptation, and the other contracture producing substances. The latter effects the two excitabilities oppositely (Singh, 1938*b*; 1939*b*). The decline of tension with continuous stimulation with A.C. can, then, be due to two possible factors: (1) adaptation, as has been described above; (2) sensitisation to ions outside that is, sodium chloride, which increase the excitability to potassium, and decreases that to A.C.

That the second factor also occurs, is shown by the following experiments :—(1) When the excitability to A.C. declines, that to potassium increases during the passage of A.C. (6 experiments); (2) the decline of tension may be diminished by decreasing the concentration of sodium chloride in the saline (Fig. 5) or increased by contracture producing substances. (3) When the muscle is stimulated continuously with A.C., the tension after

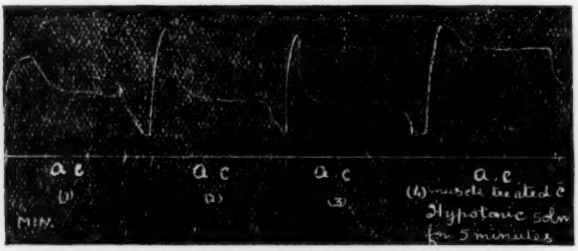

FIG. 5. *Mytilus* muscle. Stimulation with A.C. 10 V/3 min. 1st three in normal saline. 4th in Hypotonic solution containing sodium chloride, 80% of normal for 5 min.

having declined, begins to rise again. This secondary rise is due to the action of ions outside, as shown by the fact that (*a*) its relaxation is slow; (*b*) it is increased by thiocyanate and cyanide which increase the excitability to potassium and decrease that to A.C.; (*c*) it is abolished by veratrine, adrenaline which decrease tone, presumably caused by sodium chloride. (4) It is never found in frog or mammalian muscles, in which the action of sodium chloride is inhibitory. This suggests that adaptation to A.C. in these muscles is due to inhibitory action of ions in the solution, just as it is due to the antagonistic excitatory ions in *Mytilus* muscle. Further in frog muscle the excitability to potassium decreases during passage of A.C., as inhibition depresses both the excitabilities in frog muscle (Singh, 1939*b*).

The secondary rise of tension during passage of A.C. is due either (1) to direct stimulating action of ions outside or (2) to neutralisation of the factor of adaptation, producing " adaptation to adaptation " or " accommodation to accommodation " (Singh, 1943). In the former case the properties of the contraction should be that of the potassium contraction and in the latter case that of the A.C. contraction. Both these effects are found, as the tension may be increased by cyanide, thiocyanate, iodide, which increase the excitability to potassium and diminish that to A.C. or decreased by veratrine, adrenaline which decrease the excitability to A.C. and increase that to potassium.

It is probable that the first effect is to diminish adaptation, and if the action of ions is potent enough it is carried to a further stage, a tonic contraction being produced (Singh, 1942*b*).

At the end of the period of stimulation, the disappearance of the factor producing A.C. tension, should result in the A.C. off-contracture, as is experimentally found.

Adaptation due to leakage of ions.—If excitation is due to difference in concentration of ions within and without the muscle fibres (Singh, 1940) then it is to be expected that equalisation of these concentrations by diffusion of ions would produce adaptation (Singh, 1943*f*). That ions do diffuse into and out of muscle is well known. Such a possibility is suggested by the fact that calcium, the absence of which favours leakage of potassium from the fibres, may also increase the subsidence of tension during passage of A.C.; calcium thus affects the two kinds of adaptation oppositely. In the absence of calcium, the tension produced by potassium also subsides quickly; absence of calcium favours the entrance of potassium (Singh, 1938*a*). In frog muscle adaptation to ammonium is rapid compared with that to the quarternary ammonium salts, which diffuse less rapidly than ammonium (Gokhale and Singh).

The A.C. off contracture.—This has properties of the potassium contraction, so that it is produced by ions without the muscle fibres. This suggests that during stimulation ions leak out of the muscle.

The magnitude of the contracture increased exponentially with time of passage of the current (Fig. 6) and reaches its maximum when the current is passed for 20 min. and thereafter it declines; this suggests that the ions which leak out of muscle, diffuse away from the seat of action, probably into the solution. If this is so, then movement of solution in the muscle chamber might accelerate this diffusion. Out of 6 experiments, in every

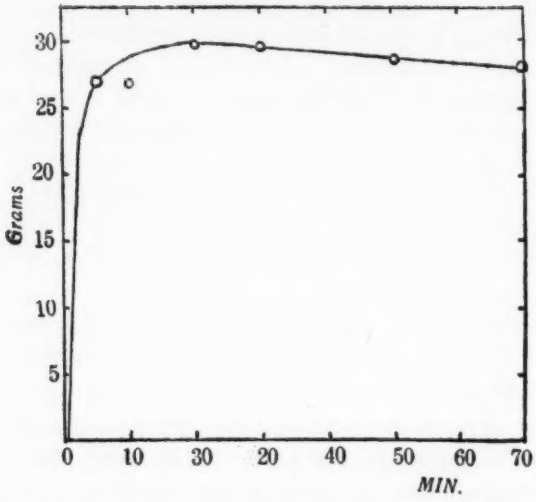

Fig. 6. *Mytilus* muscle. Effect of duration of flow of A.C. 12 V on the off-contracture

instance the magnitude of the contracture decreased if saline was made to flow at the rate of 15 to 40 c.c. per min. (Fig. 7). A drop in temperature

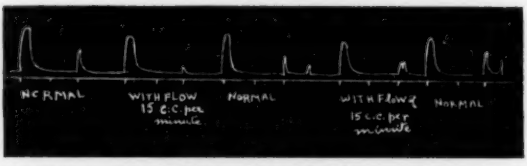

Fig. 7. *Mytilus* muscle. Effect of flow of saline upon the off-contracture

would produce the same result; the rise in temperature produced by passage of the current when there was no flow was less than a tenth of a degree as measured by inserting of thermo-couple of copper and constantan and this fluctuation is too small to affect the result. Mechanical effect of the passage of the solution should produce an opposite effect.

Equilibrium between excitation and adaptation.—In unstriated muscle " U " and " V " appear to be equilibrium. Thus if the muscle is stimulated with A.C. 8V/5 min., the tension may soon subside, showing that " U " has risen, as a consequence of the rise of " V ". If the muscle is stimulated again with 16 V, then the tension again rises only to decline to zero again. If the voltage is decreased to 8 V, then the muscle again can be stimulated with 16 V, showing that " U " had decreased as a consequence of

decrease of " V ". Ultimately, however, " V " rises higher than " U ", as shown by the fact that if continuous tension is not obtained with low voltages such as 8 V, 16 V, it may be obtained with 24 V. This shows that " U " and "V " are produced by independent agencies.

The equilibrium between " U " and " V " has a bearing on excitation at the cathode and the anode.

The fact, that on closing a current through a nerve, the excitation wave starts from the cathode, shows that cations are important agents. It is pointed out by Kieth Lucas (1912), that " the one feature which is common to the cathode when the current is made and the anode when the current has just ceased to flow, is an increase in concentration of cations above the value which occurred at each of these points immediately before ". At the anode, the concentration of cations only rises to its normal value by diffusion, after having been decreased.

The decrease in concentration of cations at the anode would diminish the adaptation factor, so that when the concentration of cations rises to its normal level, excitation takes place, as the increase in the adaptation factor is slower than increase in cation concentration.

The above view is direclty born out by experiment on the frog stomach and the guinea pig uterus. Normally the frog stomach is adapted to the ions in the saline. If the muscle is deprived of sodium by immersion in a sodium deficient solution, re-immersion in the original sodium chloride concentration causes a contraction. Thus contraction is caused when the ions, which previously were inert, were restored to their original concentration. In the guinea pig uterus, cations cause inhibition instead of contraction. The potassium normally present in the saline has no action, or the muscle slightly relaxes when it is withdrawn. If the muscle is deprived of potassium for ten minutes, restoration of the normal concentration of potassium causes marked inhibition (Singh, 1942*b*). Thus " U " and " V " are in equilibrium.

The adaptation to normal concentration of ions described above is the same as the " paradoxes " described by various authors (Libbrecht, 1920, 1921; Guerra, 1924; Witanowski, 1926; Kisch, 1927, 1930; Wells, 1928; Chao, 1934).

Effect of initial length on adaptation.—For inhibition in unstriated muscle, an optimum length is necessary (Singh, 1942*b*). As adaptation is a kind of inhibition, it would be expected that adaptation would be affected by length of the muscle. This is shown by the following experiment,

Ordinarily the isometric contraction produced by 0·1 M KCl in *Mytilus* muscle subsides in 10 to 60 min. If the muscle is allowed to shorten, the contractile process does not completely subside in 12 hours, as shown by periodically recording a stretch curve; this should not be confused with the known fact that the isometric contraction lasts longer than the isotonic one. The effect of initial length on adaptation would have a bearing on the rate of extension of unstriated muscle (Singh, 1943*b*). It would diminish tone with extension and so account for the diminished oxygen consumption if the muscle is stretched (Rao and Singh, 1940).

Another interesting feature is that if contraction is not produced with greater length, it may be produced, if the muscle is allowed to shorten

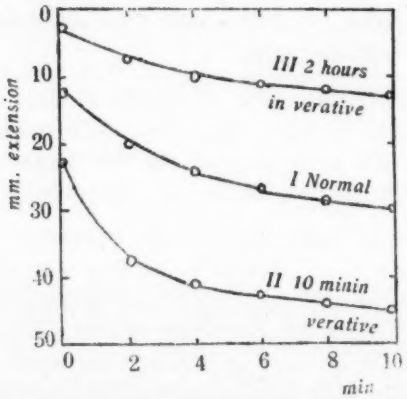

FIG. 8. *Mytilus* muscle. Stretch curves. Effect of varatrine (1 in 1000)

(Fig. 8). For this, however, the muscle is to be soaked for some time in the solution. Thus veratrine in *Mytilus* muscle and ammonium in frog muscle may produce inhibition only but if the muscle is left in the solutions for a couple of hours and allowed to shorten, a contraction results as shown by stretch curves. This phenomenon resembles that described by Parnas (1910), wherein unstriated muscle is unable to lift a weight, but if the muscle is allowed to shorten, it is then able to keep up the weight, which it was unable to lift.

In the above experiment, not only is the factor of length, but also that of time. It appears that either the factor causing adaptation leaks out of the muscle, or antagonistic substances diffuse into the fibres; ammonium and potassium are antagonistic to calcium. Small concentrations of potassium (0·02–0·05 M KCl), which are unable to evoke an isometric contraction

in *Mytilus* muscle do so if the muscle is allowed to shorten. This may occur apart from soaking. If *Mytilus* muscle is kept at rest, and allowed to shorten tone may increase together with its sodium content.

It appears that adaptation may diminish; (1) owing to decrease in initial length, (2) diffusion into the fibres of ions that are antagonistic to the factor causing adaptation, which is probably calcium. This second factor is of importance in considering diminution of adaptation to potassium and A.C.

When frog muscle is stimulated with potassium, a twitch is produced to which the muscle rapidly adapts. This twitch can be differentiated from that produced by A.C. by changes in osmotic pressure (Fig. 9). (Such a twitch

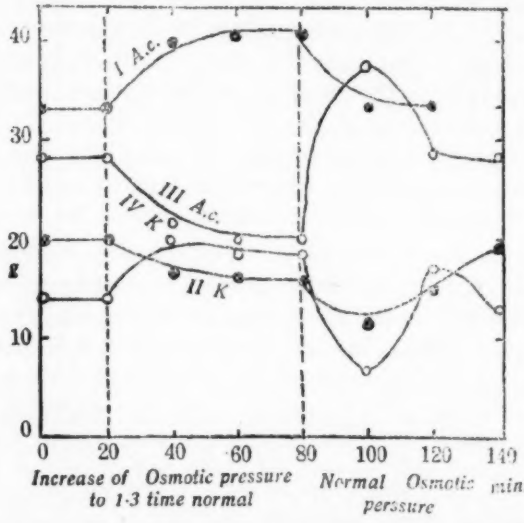

FIG. 9. Frog stomach. Effect of osmotic pressure (increased by adding sodium chloride at pH 8) on the response to A.C. 8 V/10 sec. and potassium (0·22 M).

has only been produced by A.C., potassium or ammonium and is interesting in view of the suggestion that A.C. twitch is produced by potassium within the fibres.) After the twitch, adaptation diminishes and a tonic contraction develops. With ammonium such a phenomenon is produced if preliminarily a contracture is induced by thiocyanate or iodide.

It appears that with time, the factor of adaptation has been neutralised, and as potassium and ammonium are antagonistic to the action of calcium, they probably diffuse into the muscle, and antagonise the action of the latter.

The diminution in adaptation to A.C. mentioned above is presumably due to the ions being carried into the muscle fibres by the current instead of by diffusion. The phenomenon is well marked in thiocyanate or cyanide and these are antagonistic to the action of calcium. If diminution in adaptation to A.C. is not produced with small voltages (8–16 V), it is invariably produced by higher voltages (20–40 V) the number of ions entering the muscle fibres, presumably increasing as the voltage increases; hence the rate of relaxation also diminishes.

The decline in excitability at temperature above 30° C. is due to adaptation (Rao and Singh, 1940), which is probably due to liberation of calcium. The liberation of calcium from protoplasm at high temperatures has been shown by Mazia and Clark (1938). The release of calcium would also diminish the viscosity. At higher temperatures the excitability to potassium and tone again increases. This again is probably due to neutralisation of adaptation by ions outside. The increased activity by ions outside is shown in *Mytilus* muscle by (1) increase of the A.C. off contracture; (2) increased tone; (3) increased excitability to potassium. At temperature above 37° C., the contractile mechanism is directly affected, as isolated myosin also contracts (Astbury and Dickinson, 1942).

Adaptation to direct current.—In *Mytilus* muscle, adaptation to direct current is slower than to alternating current, but in frog stomach it is more rapid. The properties of the contraction on cessation of D.C., as expected from the reasoning above, are those of the initial A.C. contraction. No contraction having the properties of the A.C. off contracture has been observed on the cessation of D.C.; this precludes the possibility of electrolysis as being the causation of the former. It therefore appears that the off contracture is associated with the reversal of the current in each wave, the ions being carried without the fibres by the reversal of the direction of the current.

The slow relaxation and the high viscosity of the D.C. contraction suggests that ions are carried with the current into the muscle fibres from without. The fact that a greater intensity of alternating current is required to produce the same effect as D.C. of lesser intensity, suggests that the only one direction of the alternating current is effective, the accumulation of ions at some place being due to rectification. The A.C. off contracture would be due to accumulation of ions external to muscle fibres, due to rectification. Delayed excitation produced by alternating current has been ascribed to rectification (Cole, 1941).

In frog stomach the inhibitory action of ions outside increases adaptation to D.C., and so decreases the primary tension. For the same reason, in the guinea pig uterus D.C. is more inhibitory than A.C.

Mytilus muscle is less excitable to D.C. than A.C. owing to the action of ions outside; an increase in calcium ion concentration may reverse this effect (Fig. 10).

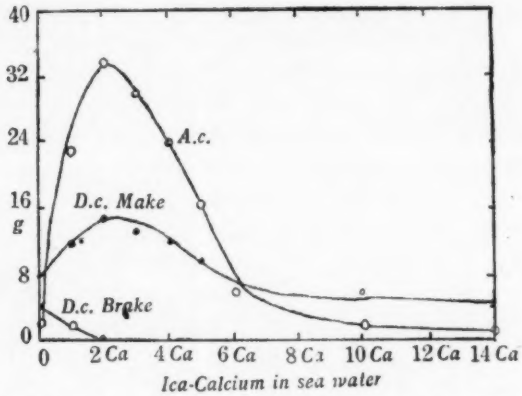

FIG. 10. *Mytilus* muscle. Effect of calcium on the response to A.C. and D.C.

In the guinea pig uterus, adaptation to A.C. inhibition is more rapid than D.C. inhibition.

Adaptation to inhibition.—There are two kinds of adaptations to inhibition; in one excitation also decreases, and in the other it increases. Adaptation to inhibition may be produced by three possible methods.

(1) Liberation of calcium. As calcium antagonises the inhibitory effect of sodium, liberation of calcium might occur. Excitatory action of ions would then also decrease. This probably occurs at high temperatures.

(2) Inhibition of adaptation. If increase in excitatory fact or "V" increases " U ", then diminution of " V " or inhibition would diminish " U "; inhibition of adaptation is shown by increase of tone. This method of adaptation to inhibition appears to be produced in majority of instances.

There is experimental support for the suggestion, that ions may suppress the adaptation factor. Mazia (1933) has found that when eggs of the sea urchin Arbacia are immersed in pure sodium chloride solutions, there is actually an increase in the free calcium concentration of the cell interior. Presumably calcium is set free from combination in the cell cortex; this effect is prevented if a trace of calcium is added to the sodium chloride solution.

(3) Penetration of ions into the interior. Just as one kind of adaptation to excitation has been presumed to take place by penetration of the ions

into the interior, a similar action is possible. The reversal of the inhibitory effect of ammonium with time may be due to penetration (*c.f.* Ing and Wright, 1931; Straub, 1903, 1907).

Inhibition of adaptation and withdrawal contractions.—The suppression of adaptation by inhibitory substances offers an explanation of the withdrawal contractions. If increase in "V" over "U" causes contraction, then excitation will also occur if "U" decreases below "V", the latter remaining constant. Thus most of the substances that produce withdrawal contractions, produced a preliminary inhibition.

The simplest explanation of a withdrawal contraction (as advanced by Straub) would be that the contraction is due to difference in concentration of ions without and within the fibres. The initial contraction would be due to the presence of ions without the fibres, and the withdrawal contraction, to their presence within the fibres. If this explanation is correct, then the initial tension should subside completely in the continued presence of the drug, and a contraction occur when the latter is withdrawn. The majority of the withdrawal contractions do not show such a phenomenon, and so the above explanation is not tenable; these contractions are tonic, and so due to ions without the fibres.

Contraction produced by withdrawal of calcium.—This contraction is clearly due to decrease of the adaptation factor, that is, calcium. In *Mytilus* muscle a tonic contraction is produced on withdrawal of calcium or potassium (0·01-0·02M). If larger concentrations, which cause contraction are used, then a withdrawal contraction is not produced; this is therefore associated with the inhibitory property of these ions. In skeletal muscle Gelhorn (1931) found that only those concentrations of thiocyanate produce a withdrawal effect which do not produce a contraction.

That the contraction produced on withdrawal of calcium is due to sodium chloride ions without the fibres is shown by the fact that in frog and mammalian muscles, the action of sodium chloride is inhibitory, and so a withdrawal inhibition is produced. Calcium thus antagonises the action of sodium or chloride. In the guinea pig uterus, a withdrawal contraction or inhibition is produced depending upon the action of sodium chloride (Singh, 1942*b*, 1943*g*).

In the guinea pig uterus, though withdrawal of calcium, normally present in the saline does not produce contraction, large concentrations that produce inhibition produce a contraction on withdrawal (Fig. 11). This suggests that excess of calcium suppresses the liberation of calcium. It appears that calcium exists in some combination with protoplasm; in this

combination it is inactive. There further appears to be some relation between the combined and uncombined calcium; if there is too much of

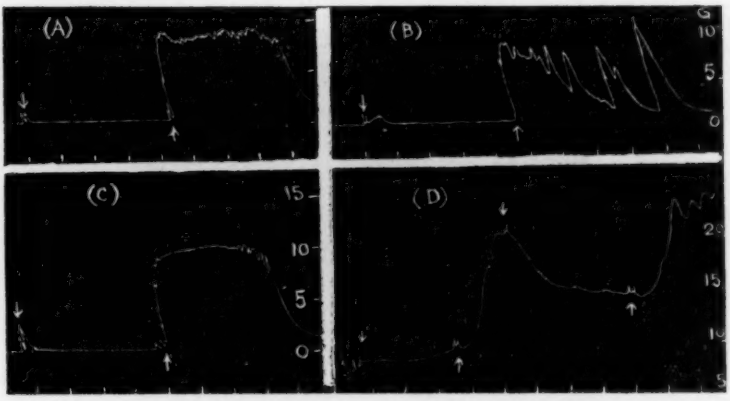

FIG. 11. Guinea pig uterus. Withdrawal contractions: (A) With drawal of 0·03 M $CaCl_2$ added at ↓ and withdrawn at ↑; (B) Withdrawal of 0·05 M $CaCl_2$; (C) Withdrawal of 0·05 M $SrCl_2$; (D) Withdrawal of adrenaline, 1 in 10^5 (dog stomach).

the latter it goes into combination, a relation similar to that which would be produced by the law of mass action.

At the equilibrium point there is a definite relation between the concentrations of ethyl alcohol, acetic acid and etyhl acetate. If more ethyl alcohol is added then more ethyl acetate will be formed. If now by some process we are suddenly able to withdraw the ethyl alcohol that was secondarily added, there would be left behind a disproportionately less amount of ethyl alcohol than was present in the beginning.

Similarly, if a compound between calcium and protoplasm is governed by similar relations, then if the concentration of calcium is increased more of it would go into combination, and if the excess of calcium is withdrawn, there would be a deficiency of ionised calcium and so a tonic contraction produced.

Liberation of calcium from protoplasm is known to occur in a variety of conditions (Heilbrunn, 1937, for reference). From the above process of reasoning, withdrawal of calcium should result in liberation of calcium, as has been shown by Mazia (1933).

The recombination of calcium appears to be produced not only by calcium, but also by allied ions such as strontium, the withdrawal of which causes a contraction (Fig. 11).

Contraction produced on withdrawal of ammonium.—(Singh, 1939*b*). Ammonium produces the following kinds of withdrawal contraction :— (1) A contraction which completely subsides, occurs on addition of ammonium; on withdrawal a further contraction occurs (Fig. 12). As there is

FIG. 12. Frog stomach. Contraction produced by addition (X) and withdrawal (Y) of 0·02 M NH_4Cl.

no preliminary inhibition, the withdrawal contraction could not be due to suppression of the adaptation factor. It can be explained on the basis of penetration, of ammonium ions into the muscle fibres. As the initial contraction subsides in a minute or two, the response must be of the surface fibres only (Singh, 1943*f*), and equilibrium must be rapid, the ions probably just crossing the membrane; the rapid subsidence may also be due to the liberation of the adaptation factor, so that in this case, there is probably excess of the latter.

The above view is supported by chemical analysis. In frog stomach, ammonium in concentration used to produce the withdrawal contraction replaces potassium within the fibres (Gokhale and Singh); besides the properties of the contraction are that of the A.C. contraction. The muscle is less permeable to quarternary ammonium salts, and they do not produce such a contraction. In dog stomach, there is no appreciable difference between the potassium contents of muscles, soaked in ordinary saline, and saline containing ammonium in concentration, used for production of the withdrawal contraction, and such a contraction has not been observed (Gokhale and Singh).

(2) The initial contraction on addition of ammonium may be absent, or only a preliminary inhibition is produced. In frog stomach this preliminary inhibition is increased if the sodium chloride of the saline is decreased by 20 p.c.

(3) A preliminary inhibition occurs, but the muscle recovers from the inhibition, and may even contract. On withdrawal there is a tonic contraction (Fig. 13).

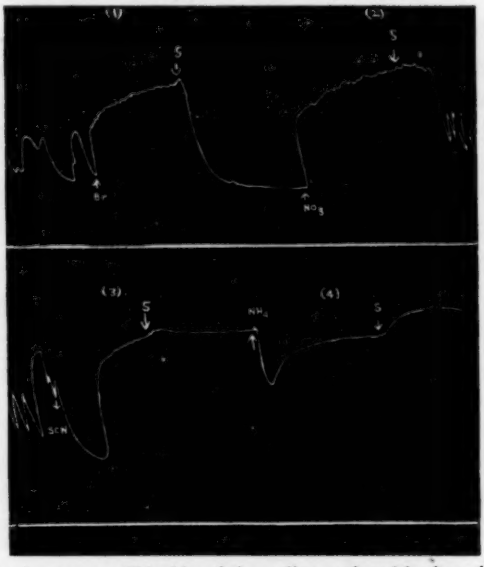

FIG. 13. Guinea pig uterus. Chloride of the saline replaced by bromide at Br, withdrawn at S. Nitrate added at NO_3, thiocyanate at SCN, and ammonium at NH_4 and respectively withdrawn at S.

The recovery from inhibition, and the subsequent contraction, shows that the adaptation factor has been suppressed as mentioned above. The phenomenon is very important showing that excitation releases the adaptation factor and inhibition suppresses it.

In the guinea pig uterus, eserine aids the suppression of the adaptation factor, as with adrenaline the preliminary inhibition is followed by a contraction and on withdrawal another contraction occurs (Agar, 1940) with the exception of the withdrawal contraction, a similar action is produced by deficiency of sodium chloride in the case of potassium (Singh, 1942*b*).

Contraction produced on withdrawal of potassium.— There are two kinds of contractions, one produced by withdrawal of small concentrations in *Mytilus* muscle, and the other by large concentrations in the guinea pig uterus. The former contraction is akin to the calcium withdrawal contraction, potassium having a calcium-like action. The latter contractions are of two kinds: (1) like those produced by withdrawal of ammonium; (2) like

those as shown in Fig. 4 of Singh (1943*g*). There is no preliminary inhibition, but contraction on addition as well as withdrawal of potassium, like the D.C. contraction in *Mytilus* muscle. For this contraction, no explanation is satisfactory, except that the preliminary inhibitory phase may be regarded as transient or latent.

The contraction described above on withdrawal of potassium is a tonic contraction as shown by the following considerations. On frequent stimulation the initial contraction is replaced by inhibition, and on withdrawal of potassium, the usual re-development of tone occurs; the original contraction has disappeared, but the withdrawal contraction remains in the above form.

That potassium should cause inhibition or contraction in the same muscle, shows that the factor determining inhibition or contraction resides in the cell. It is possible to antagonise one of these, and so produce the other result.

Contraction produced on withdrawal of anions.—The preliminary inhibition by thiocyanate is well marked (Fig. 13). In *Mytilus* muscle, with sulphate there is no inhibition, this is probably due to changes in the calcium concentration or the adaptation factor.

Contraction on withdrawal of drugs.—The preliminary inhibition is well emphasised; adrenaline and acetylcholine produce a withdrawal contraction in *Mytilus* muscle only if the muscle is insensitive to these drugs. In dog stomach, and the guinea pig uterus, the preliminary inhibition is proded by adrenaline. In *Mytilus* muscle, veratrine produces withdrawal contraction only if the preliminary contraction is little marked (at pH 6·5) or is abolished by A.C.

In guinea pig uterus inhibitory substances produce a withdrawal contraction, and excitatory ions like chloride, bromide, acetylcholine produce a withdrawal inhibition. The former is probably due to inhibition and the latter to liberation of the adaptation factor; the latter is identical with the inhibition of tone found just after stimulation. This inhibition of tone really results during stimulation, and outlasts the latter.

A withdrawal stimulus may not actually produce a contraction, but may retard the relaxation of the contraction like veratrine in skeletal muscle. This is shown by Fig. 13. Bromide produces no withdrawal contraction, and the relaxation of the bromide contraction is rapid. Nitrate, thiocyanate and ammonium produce withdrawal contractions, the potency being in the order, $NO_3 < SCN < NH_4$; relaxation is also retarded in the same order.

Slow relaxation after chemical stimulation is also due to passive changes probably increase in viscosity. This is suggested by the fact, that the contraction persists though the stimulating substance is withdrawn, the muscle relaxing slowly, alike in the presence or absence of the stimulus.

Thus after chemical stimulation, as after electrical stimulation, the slow relaxation may be due to an active contraction, or may be a passive phenomenon, due to structural changes in the muscle.

Fatigue.—Fatigue with intermittent stimulation and adaptation with continuous stimulation are identical; the former is due to the persistence of the adaptation factor. Hence, as there are two kinds of adaptation, there will be two kinds of fatigue. In one kind of fatigue, the excitability to potassium will decrease, and in the other kind, it will increase.

The two kinds of fatigue to A.C. are beautifully shown in Figs. 1 and 2. In the former the A.C. off contracture, hence the sensitivity to ions without increases, and in the latter, it decreases. The former occurs in potassium sensitive muscles, and the latter in comparatively insensitive muscles. The former fatigue is due to increased sensitivity to ions outside so that contracture producing substances, such as adrenaline (1 in 10^5), convert the first kind of fatigue into the second kind, by antagonising the factor of adaptation, and increases the second kind of fatigue (see Fig. 5, Singh, 1939*b*).

As mentioned above, the neutralisation of adaptation is probably due to entrance of ions from the solution into the fibres. The entrance of ions would account for the different effects produced by change of the direction of direct current on fatigue (Heilbrunn, 1937, for references). As anions are antagonistic to A.C. the accumulation of cations at one end of the muscle fibres will be antagonised by the anions; at the other end the fibre will be poorer in anions, and when the current is reversed, the excitability will be greater.

The above experiments clearly show that fatigue could not be due to consumption of substances responsible for contraction, but it can be due to accumulation of waste products if the latter increase the permeability and thus make the muscle more sensitive to action of ions without.

The increase of fatigue at high temperatures are also due to the same two factors, *viz.*, increase in adaptation. The second factor also comes into play, as the excitability to ions also increases. As these two factors are antagonistic, the result will depend on their interaction. Variable results of temperature have been obtained by Bernstein (1908) and others.

It is evident that calcium will affect the two kinds of fatigue oppositely.

Beneficial effect of contraction.—This is produced by the liberation of the adaptation factor in potassium sensitive *Mytilus* muscle, as this is antagonistic to the action of ions outside; in potassium insensitive muscles, the same factor will produce fatigue. The above conclusion is supported by the fact that in potassium insensitive muscles the beneficial effect of contraction is marked if the concentration of calcium is decreased (Fig. 4), thereby increasing the action of ions outside. It is also supported by the fact that the rate of relaxation increases so that bigger the contraction, the more quickly it relaxes, the slow relaxation being due to the ions outside (Singh, 1938*e*). This is the reason why the contractions cannot be superposed on each other (Bozler, 1930; Ritchie, 1931), and neutralisation of the tonic contraction in freshly dissected muscle by stimulation (Bayliss, Boyland and Ritchie, 1930). As with fatigue, the two excitabilities may be similarly or oppositely affected.

In frog muscle, the beneficial effect of contraction is due to antagonising the inhibitory action of ions outside, instead of excitatory, as in *Mytilus* muscle.

Recovery of excitability.—The liberation of the adaptation factor would result in a stage of hyper-excitability during recovery; this conclusion is supported by the fact that in potassium insensitive muscles, the stage of hyper-excitability is absent. As the ions outside have not much effect, on recombination of the adaptation factor, there will be no depression of excitability. The beneficial effect of contraction and the supernormal phase during recovery are thus due to similar causes, and are so affected identically (Adrian, 1921); for the same reason they are most marked in the same muscle.

Relaxation.—The muscle adapts at different times to slow relaxation and to the primary A.C. tension, so that these are produced by two distinct agencies relaxation is slow if the current is continued for 3-4 seconds, and becomes rapid thereafter, but the primary tension continues to increase. The primary A.C. tension is produced by ions within and the slow relaxation by ions without the fibres.

The slow relaxation with stimuli of short duration would thus be due to lack of adaptation as " V " rises more rapidly than " U ". As the action of ions outside would increase with increasing voltage, the slow relaxation at higher voltage would be due to neutralisation of adaptation or " adaptation to adaptation ".

The secondary A.C. contracture.—The liberation of the adaptation factor during stimulation with A.C. would diminish tone; when the adaptation

factor recombines, the ions outside would exert their action, producing a tonic contraction. The muscle would, however, readapt, thus producing the secondary A.C. contracture.

Magnesium shortens the latent period of the secondary A.C. contracture (Singh, 1938*b*), so that, like other inhibitory ions, it suppresses adaptation, or makes the fall of " U " quicker.

Inhibition.—There are two kinds of inhibition (1) external inhibition or by ions without the fibres; (2) internal inhibition or that produced by electric current and increase of osmotic pressure, that is, ions within the fibres. There are several kinds of external inhibition (*a*) tone diminishes, the excitability to A.C. as well as potassium is increased; this is produced by eserine, 1 in 200,000 in *Mytilus* muscle; (*b*) tone diminishes, the excitability to A.C. is increased and that to potassium decreased; this is produced by calcium in *Mytilus* muscle; (*c*) tone diminishes, the excitability to A.C. is decreased and that to potassium increased this is produced by veratrine in *Mytilus* muscle in acid solutions; (*d*) tone diminishes, the excitability to A.C. as well as potassium is decreased. This is produced by adrenaline in frog stomach.

The above shows the complicated nature of inhibition. When tone is diminished, and the excitability to A.C. as well as potassium is increased, it may be inferred that the substance favours action of cations, and suppresses that of anions, tone presumably in the case being due to chloride. When tone is diminished, the excitability to A.C. is decreased and that to potassium increased, it may be inferred that the substance favours the action of cations outside and suppresses that of anions, tone again presumably due to chloride. In case (*d*) above, both anions as well as cations are suppressed. In case (*b*) the action of ions outside is suppressed.

In frog stomach inhibition affects both the actions of A.C. as well as potassium identically, but in *Mytilus* muscle the action of A.C. is enhanced. This latter result is probably an indirect effect, produced by antagonising the action of ions outside. In frog stomach this does not occur, as the sodium chloride content of the saline is low, but can be made to occur by enhancing the stimulating power of the ions of the frog saline replacing part of the sodium with potassium. Inhibition, therefore, like adaptation, affects the two excitabilities identically.

When the various actions described above are analysed, tone appears to be caused by chloride.

Antagonism between sodium and chloride.—When unstriated muscle is immersed in isotonic solution of sucrose of glucose, a contracture develops.

In frog stomach, guinea pig uterus and *Mytilus* muscle, introduction of sodium chloride causes inhibition ; this shows that sodium chloride contains an inhibitory factor. In frog stomach contraction may ensue showing that it also contains an excitatory factor. In guinea pig uterus, after adaptation sodium chloride causes contraction, the inhibitory factor being antagonised by adaptation; thus, here too sodium chloride contains an excitatory factor.

In *Mytilus* muscle sodium chloride deficiency in the saline may either increase or decrease the excitability to A.C. inhibitory cations in the *Mytilus* saline, such as calcium and potassium increase the excitability to A.C. If the chloride of the saline is replaced with excitatory ions such as bromide, nitrate, iodide, then their partial removal always increases the excitability to A.C. This shows that in *Mytilus* muscle increase in excitability in sodium chloride deficient solutions, must be due to removal of the chloride, and decrease to removal of the sodium. Thus the actions of sodium and chloride are antagonistic.

In frog stomach small concentrations of anions increase the excitability by antagonising the inhibitory action of cations, larger concentrations decrease the excitability as in *Mytilus* muscle owing to their excitatory action.

Plain muscle exhibits two kinds of tone: (1) by ions within the fibres, (2) by ions outside the fibres (Singh, 1939*b*). Tone produced by ions without, again, is of two kinds; (1) with high viscosity, (2) with low viscosity (Singh, 1943*a*). In guinea pig uterus cations in small concentrations produce the tonus with low viscosity. It therefore appears that tonus with high viscosity is due to anions, and that with low viscosity to cations; chloride and sodium probably produce these two kinds of tone respectively.

Discussion

There are two kinds of adaptation to excitation; one is slow and the other quick. The quick adaptation is probably brought about by liberation of calcium. If the length of the muscle is decreased, then the quick adaptation does not seem to occur, and the adaptation that occurs after prolonged soaking appears to be due to diffusion of ions into the interior of the muscle fibres.

The equilibrium between " U " and " V " suggests liberation of calcium by ions.

That practically all known types of stimulating agents can cause release of calcium from protoplasm is shown by the work of Mazia and Clark

(1936). They were able to show that ultraviolet radiation, electric shock, mechanical impact, heat hypertonic solutions all caused a release of calcium from the protoplasm.

The liberation of calcium assumed to take place during A.C. stimulation (Singh, 1938*b*, 1939*b*, 1940) is probably also be due to the same factor, that is increase ionic concentration; this is the reason that " U " rises as well as declines more slowly than " V ".

If adaptation is brought about by the action of ions outside, then the excitability of the muscle to potassium should increase during the passage of the current as the ions outside increase the excitability to potassium (Singh, 1938*b*). If adaptation is brought about by the second factor, that is increase in the calcium ion concentration, then the excitability to potassium should decrease.

There is a relation between the permeability of the muscle and excitability. If adaptation is brought about by ions outside, then the permeability should increase; if by calcium, it should decrease. Decrease in membrane resistance with excitation indicating increased permeability, has been found by Duboisson (1934), Ebbeck (1932), McClendon (1912, 1927), Ray and Rapport (1927), Buchthal (1934), Hogben and Gordon (1930), Achelis (1932). Bozzler, Bozzler and Cole (1935) found an increase in the resistance or impedance of the muscle, Hartree (1935) found no change. Variable results are to be expected.

Noonan, Fenn and Haege (1941) found that in frog skeletal muscle, there was no increase in permeability to radio-active potassium during electrical stimulation of frog skeletal muscle. This is in agreement with the views mentioned previously, that electrical stimulation is due to ions within the fibres; no increase in permeability would be necessary. As mentioned above, a decrease of permeability should be expected.

In plain muscle, too, there should be a decrease in permeability, though this should be preceded by a transient increase, according to the sensitivity to ions without (Singh, 1938*e*). In *Mytilus* muscle however, the decrease of permeability should be followed by an increase if it shows a tonic contraction on electrical stimulation. Similarly with chemical stimulation, a decrease of permeability should occur if adaptation occurs; otherwise an increase should occur. In diffusion experiments these opposing tendencies might neutralise each other.

With nervous stimulation, these changes are to be expected, if the mechanism of stimulation is the same.

A decrease in the concentration of potassium within the fibres, should produce tonic results, such as slowing of the rate of relaxation or increase in viscosity, with frequent stimulation. Both these changes are known to occur.

Summary and Conclusions

(1) There are two kinds of adaptation to alternating current. During one, the sensitivity to potassium decreases, and during the other, it increases. One is probably produced by calcium, and the other by ions outside.

(2) There are two kinds of adaptation to chemical stimulation; one is similar to the first kind of adaptation to A.C. and the second one is probably due to diffusion of ions into the muscle fibres. This kind of adaptation also occurs to A.C.

(3) Adaptation may be diminished by (*a*) leakage of the adaptation factor, (*b*) neutralisation of the same or (*c*) or inhibition of adaptation. This last appears to be responsible for tonic withdrawal contractions.

(4) Adaptation increases with length.

(5) Adaptation and fatigue are identical.

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A CONTRIBUTION TO THE LIFE-HISTORY OF *SONERILA WALLICHII* BENN.*

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(Communicated by Prof. L. Narayan Rao, Ph.D., F.A.Sc.)

In continuation with the embryological studies on some of the members of the *Melastomaceæ*, the writer has been able to obtain some interesting facts on the life-history of *Sonerila Wallichii* Benn. which is presented here. A survey of literature indicates that the genus *Sonerila* has received less attention in the hands of the plant embryologists than other genera of *Melastomaceæ*. Ziegler (1925) has given a brief account of the floral morphology of *Sonerila Leopoldii* Hort. particularly with reference to dehiscence of anthers and other details. An account of the megasporogenesis of *Sonerila speciosa* Zenk. has recently been published by George (1938).

The plants of *Sonerila Wallichii* for the present studies were collected in Agumbe, Mysore State. Characteristic pink flowers arranged in subumbellate cincinni are very characteristic of the plants.

The stamens are three in number with quadrilocular anthers. The structural details of the wall of the mature anther cannot be clearly observed owing to the fact that the wall layers get disorganised contributing nutrition to the developing megaspores. However, the wall of the anther (Fig. 1) consists of an epidermal layer, endothecium which is ill-developed, a single middle layer and an innermost tapetum, the cells of which remain uni-nucleate. The microspore mother cells derived from the sporogenous tissue undergo the usual meiotic divisions producing tetrads of microspores. Chromosome counts made in the metaphase plates revealed eight chromosomes, the number however needs to be confirmed in sections of the root tips. Mature pollen grains are ovate to subglobose and show three-nucleate condition (Fig. 2).

The ovary is semi-inferior and is covered by a persistent hairy calyx. The ovary is trilocular and the anatropous ovules, several in each loculus and bitegumentary are arranged on an axile, slightly projecting placenta. The integuments are two layered. They grow beyond the nucellus, and their free

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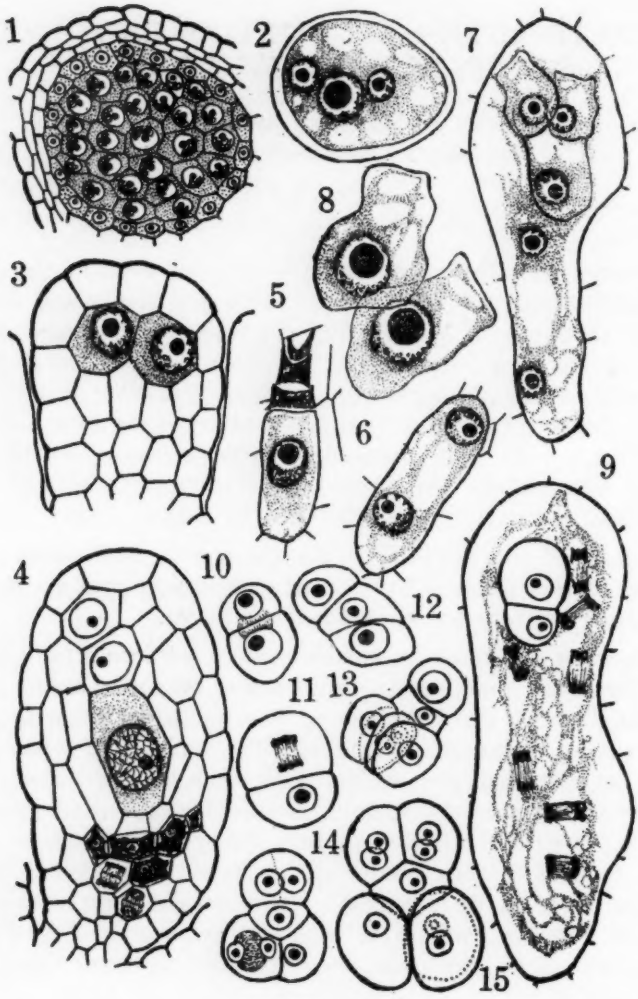
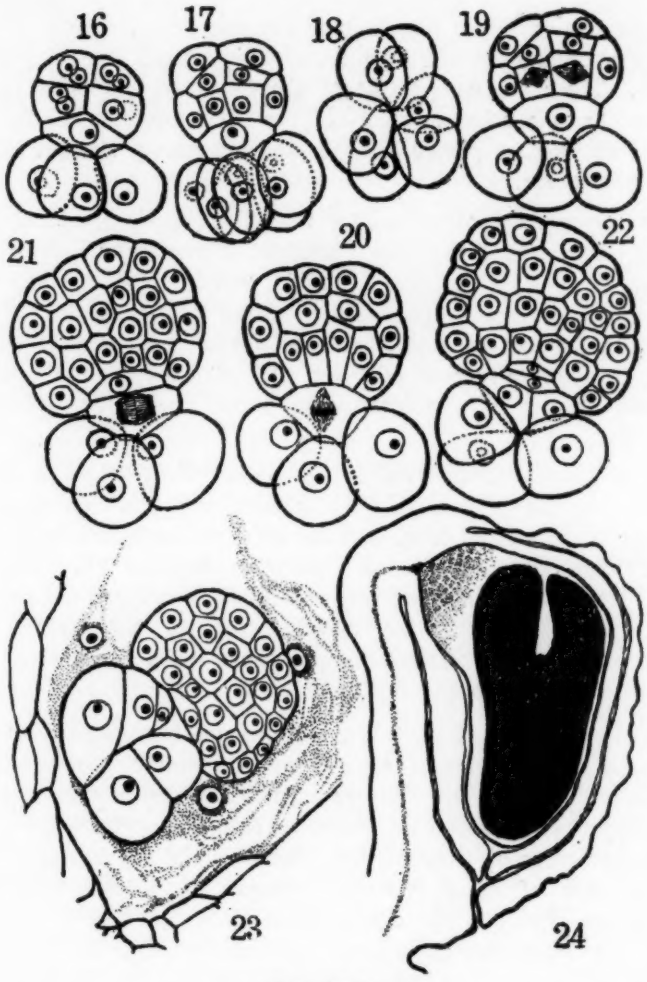


FIGS. 1–15

Fig. 1. Transverse section of an young anther showing wall layers. ×540. Fig. 2. Mature pollen. ×1800. Fig. 3. Multiple archesporium. × 1800. Fig. 4. Megaspore mother cell. ×1800. Fig. 5. Linear tetrad showing three degenerating megaspores and the enlarging chalazal megaspore. ×1800. Fig. 6. Two-nucleate embryo-sac. ×900. Fig. 7. Mature 8-nucleate embryo-sac. ×900. Fig. 8. Egg-like synergids with apical vacuoles. ×1800. Fig. 9. Two-celled pro-embryo with free nuclear endosperm. ×900. Fig. 10. Two-celled pro-embryo. ×900. Figs. 11 and 12. Stages in the formation of three-celled pro-embryo. ×900. Figs. 13–15. Stages in the development of embryo. ×900.



FIGS. 16-24

Figs. 16–22. Stages in the development of embryo. ×900. Fig. 18. The transverse section of the suspensor showing plate of six cells. ×900. Fig. 23. Developmental stages of incipient multiple embryos. ×900. Fig. 24. Showing the mature dicot embryo with chalazal strands in the ovule. ×240.

ends are slighlty dilated. The micropyle is formed by both the integuments and is not so zig zag as in the other members of the Melastomaceæ (Subramanyam, 1942). As in *Sonerila speciosa* (George, 1938) the inner integument is rather thin and is crushed during development. The nucellus is rather massive and is nearly straight.

At the region where the chalazal vascular strand enters the ovule a group of nucellar cells with rich cytoplasmic strands can be differentiated (Fig. 24). These are comparable to the hypostase-like tissue recorded by the writer for the other members of the Melastomaceæ (1942). What is described as nucellar glandular tissue in *S. speciosa* (George, 1938) might refer to this tissue.

The hypodermal archesporium is differentiated simultaneously with the inner integument. Sometimes a multiple archesporium is present (Fig. 3) consisting of two or three cells. Double tetrads were also met with, and in this respect it resembles *Leandra cordifolia* (Subramanyam, 1942). The archesporium divides by a periclinal wall forming the primary parietal cell and the meagaspore mother cell (Fig. 4). As development proceeds, the megaspore mother cell becomes deep seated due to the divisions of the parietal cell.

The megaspore mother cell in the course of its development enlarges in size by crushing the nucellar cells surrounding it. It undergoes the usual heterotypic divisions and forms a linear tetrad of megaspores (Fig. 6). The chalazal megaspore enlarges and develops further. Simultaneous development of the chalazal megaspore as well as the second one has been noticed in many cases similar to the condition recorded by the writer (1942) in *Leandra cordifolia* and *Osbeckia hipidissima*. As in *Osbeckia Wightiana* the upper two megaspores degenerate first.

The development of the functioning chalazal megaspore conforms to the normal type. Mature embryo-sac becomes elongated and slightly dilated at the tip (Fig. 7). The egg apparatus which becomes organised at the micropylar end consists of somewhat elongated synergids with pointed tips. Similar types of synergids have been noticed by George (1938) also. The occurrence of apical vacuoles recorded for other members of the *Melastomaceæ* (1942) is the characteristic feature of the present species (Fig. 8). The antipodals show early degeneration. The entry of the pollen tube is of the porogamous type. The endosperm is free nuclear, the nuclei dividing prior to the fertilized egg. Some of these division stages have been figured (Fig. 9). The endosperm nuclei thus formed become placed in peripheral position. When compared with the other members of the Melastomaceæ, the endosperm nuclei are not so large in number.

During post-fertilization stages, the nucellar cells at the chalaza break down contributing nutrition. The embryo-sac portion also dilates abutting on the degenerated cells. A definite chalazal haustorium is described by George (1938) in *S. speciosa* which according to him digests the glandular

tissue (hypostase tissue ?). It was however noticed by the writer that even before the chalazal end of the embryo-sac dilates, the nucellar cells in that region disorganise. It is doubtful whether any haustorial function be attributed to the chalazal end of the embryo-sac.

The embryo in its development conforms to the *Capsella*-type. The first division of the zygote is transverse and a two-celled proembryo is formed. The upper cell divides again resulting in the formation of a filamentous row of three cells (Figs. 11 and 12). In the case of *L. cordifolia* and *O. hispidissima* the proembryo consists of 5 cells. The chalazal cell develops into the embryo and even prior to any activity on the part of the embryonal cell, the micropylar cell enlarges and divides by vertical wall formation resulting in the production of a longitudinal group of six cells (Figs. 13, 14 and 17). A transverse section at the micropylar region reveals a plate of six cells (Fig. 18).

The terminal embryonal cell begins to assume a more or less spherical shape and divides into quadrants and later on into octants (Figs. 13, 14, 15 and 16). The four upper octants form the stem and cotyledons and the four basal ones the hypocotyl, except its tip. In the octant stage the dermatogen is cut off by periclinal walls and the periblem and plerome are formed afterwards (Figs. 17 and 19). The other histogens are completed by the hypophysis which in the present form is the transformed middle suspensor cell (Figs. 20, 21 and 22). The adult embryo is dicotyledonous and the cells of the embryo contain large quantities of deeply staining reserve materials (Fig. 24).

Occasional tendencies towards the formation of multiple embryos due to the activity of the suspensor cells have been noticed (Fig. 23). Even though their development to any appreciable extent have not been observed, their tendency to divide and form a group of cells is however significant. The nucellar polyembryony observed by the writer (1942) in *Osbeckia hispidissima* has not so far been noticed in any other member of the Melastomaceæ. The division of some of the basal suspensor cells upto the three-celled stage might only be cases of arrested development. Nutritional factors might play an important rôle in these cases.

Summary

An account of the life-history of *Sonerila Wallichii* with reference to microsporogenesis, megasporogenesis and embryogeny is presented in this paper. The chromosome number as determined in the pollen mother cells is eight. The ovules are bitegumentary with mostly a single and rarely

multiple archesporium. Further developmental stages of the multiple archesporium to double tetrad stages have been noticed. A hypostase-like tissue at the chalazal region is prominently seen. The development of the embryo-sac conforms to the normal 8-nucleate type.

Endosperm is nuclear and the type of development of the embryo is akin to the *Capsella*-type. The basal cell of the suspensor divides by vertical walls and forms a vertical group of six cells. Some of these might further develop into groups of cells resembling multiple embryos.

The writer wishes to acknowledge his indebtedness to Dr. L. N. Rao, Professor of Botany, University of Mysore, for guidance and encouragement and to Mr. M. J. Thirumalachar for helpful suggestions.

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BLOOD UREA CLEARANCE OF MYSOREANS

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The importance of blood urea clearance test is well known. As even slight grades of impairment of kidney function are indicated by the test, it is carried out as a routine diagnostic measure in all cases of suspected renal disease. Osgood[5] states that it is more sensitive than the blood urea nitrogen or phenolsulphonaphthalein tests, and that it has no contra-indications as have the dilution and concentration tests. The preliminary preparation of the subject is not elaborate. It has the additional advantage of including the estimation of blood urea.

The clearance values are usually determined in a large number of normal subjects and the values so obtained are used as a basis of comparison in interpreting similar results obtained in diseased conditions. Moller *et al.*[4] have found that urea clearance varies in direct proportion to the body surface. The average maximum (C_m) and standard clearance (C_s) of healthy American subjects with a mean body surface of 1·73 m^2 is 75 c.c. and 54 c.c. respectively. It is customary to express the C_m or C_s value of a given subject as percentage of these normal figures. Thus an American with a C_m of 44 c.c. has a maximum clearance of 59% and one with a C_s of 39·2 c.c., a standard clearance of 72·5%. Undue significance is not attached to small variations of clearance values as the normal range itself varies widely. Van Slyke[2] interprets the clearance test as follows :—

Percentage of average normal function	Renal function
Over 70	Normal
70–40	Mild deficit
40–20	Moderate deficit
Below 20	Severe deficit
Below 5	Ureamic coma present or imminent

Applying the above standard, an Indian with a C_m of 44 c.c. which is about 59% of the American average, has a mild renal deficit; but according

to Gokhale[1] 44 c.c. for C_m is the normal average value for healthy Indian subjects, the average C_s value being 33·8 c.c. Gokhale has come to the conclusion that the urea clearance figures of Indians should not be interpreted in terms of American or European standards as the Indian standard is different. The disparity in C_m and C_s standards of American and Indian subjects roused our curiosity and this investigation was undertaken to find out if such a difference existed among Indians living in different parts of the country. Gokhale's subjects hailed from the Bombay Presidency. We have chosen subjects from Mysore for our investigation.

The technique of the test followed by us was the same as that of Gokhale, but not the method of urea estimation. We have made use of the xanthydrol micro method as modified by Lee and Widdowson.[3] These authors have discussed the comparative merits of the different methods of urea estimation in biological fluids and recommended the xanthydrol method for research purposes especially for urea clearance.

We chose for our investigation 61 men from the Medical College, 57 of whom were students and 4 attenders. The majority of the students were residents of hostels in the city. 48 amongst them were vegetarians and the remaining 9 lived on a mixed diet. The vegetarian diet consisted of rice, dal, vegetables, milk and milk products, and the mixed diet included meat occasionally. The test was carried out during the months of November and December 1942, and all the subjects were in normal health.

The object of the test was explained to all the subjects, and they were told how they should co-operate if it should be a success. After the usual meal on the previous night, they came to the laboratory in the morning at 7-30 on an empty stomach and rested for ½ hour. Then they completely voided the urine. After complete evacuation of the bladder they were given 400 c.c. of distilled water to drink and the time at which water was drunk noted. Then the subjects remained at rest for the next 2 hours. Urine was collected at the end of 1st and 2nd hours and the volumes measured. Ten minutes before the end of the 1st hour the subject's finger tip was pricked and 0·2 c.c. of blood drawn. The blood and urine were immediately used for urea estimations. At the end of the 2nd hour, before the subjects left, their height and weight were recorded. A standard solution containing 6 mg.% of chemically pure urea was prepared for colour estimations. 0·5 c.c. of this solution was used for blood urea and also for urine urea, when the volume of urine was large and pale in colour; and 1·0 c.c. when the urine was highly coloured and small in volume.

The results of the investigation are embodied in Tables I, II and III, the values of the attenders being discarded. 31 students passed 2·0 c.c. or more

TABLE I

C_m values during the two consecutive hours

Number of the subject	Age in years	Surface area in m^2	B Blood urea mg. per 100 c.c.	V Vol. of urine c.c. per minute	U Urine urea mg. per 100 c.c.	Maximum clearance $\frac{UV}{B}$	
3*	19	1·45	24·99	3·16	409·2	51·84	1st hour
				2·66	499·8	53·32	2nd ,,
4	20	1·44	23·70	2·66	499·8	56·23	1st ,,
				2·16	562·8	51·44	2nd ,,
5	23	1·40	24·99	6·00	204·6	49·12	1st ,,
				5·00	204·6	40·94	2nd ,,
6*	21	1·72	22·50	3·16	409·2	57·58	1st ,,
				3·00	409·2	54·55	2nd ,,
9	22	1·54	28·14	4·50	375·0	59·96	1st ,,
				4·16	375·0	55·51	2nd ,,
14	22	1·56	23·70	3·33	409·2	57·54	1st ,,
				3·83	310·2	50·18	2nd ,,
15	22	1·67	22·50	4·00	249·9	44·42	1st ,,
				2·16	499·8	48·11	2nd ,,
17*	24	1·50	24·99	3·83	300·0	46·01	1st ,,
				4·16	375·0	62·50	2nd ,,
18	23	1·65	30·00	5·66	204·6	38·65	1st ,,
				2·33	409·2	31·82	2nd ,,
19	24	1·50	30·00	3·01	300·0	30·16	1st ,,
				2·83	300·0	28·32	2nd ,,
20	23	1·53	23·70	3·86	204·6	33·38	1st ,,
				3·83	204·6	33·09	2nd ,,
21	23	1·70	32·13	4·33	300·0	40·46	1st ,,
				2·73	499·8	42·51	2nd ,,
25	23	1·48	22·50	2·23	409·2	40·61	1st ,,
				3·00	391·2	52·16	2nd ,,

TABLE I—(*Contd.*)

Number of the subject	Age in years	Surface area in m^2	B Blood urea mg. per 100 c.c.	V Vol. of urine c.c. per minute	U Urine urea mg. per 100 c.c.	Maximum clearance $\frac{UV}{B}$	
27*	31	1·53	28·14	3·83	300·0	40·86	1st hour
				3·63	237·0	30·62	2nd ,,
28	22	1·51	30·00	4·00	321·6	42·88	1st ,,
				3·80	281·4	35·62	2nd ,,
29	24	1·53	23·70	2·63	333·6	37·07	1st ,,
				2·30	333·6	32·38	2nd ,,
30	22	1·50	34·62	5·70	204·6	33·67	1st ,,
				3·16	360·0	32·93	2nd ,,
32	28	1·48	22·50	2·46	409·2	44·85	1st ,,
				3·00	300·0	39·99	2nd ,,
33	24	1·43	26·46	4·93	310·2	57·84	1st ,,
				4·33	310·2	50·81	2nd ,,
34*	21	1·74	34·62	4·58	310·2	41·06	1st ,,
				4·50	375·0	48·73	2nd ,,
35*	19	1·47	26·46	5·00	281·4	53·18	1st ,,
				2·00	600·0	45·35	2nd ,,
36	23	1·61	28·14	3·30	333·6	39·12	1st ,,
				3·26	346·2	4C·20	2nd ,,
37	19	1·21	30·00	5·16	281·4	48·46	1st ,,
				4·10	346·2	47·32	2nd ,,
38	20	1·60	28·14	3·06	455·0	49·04	1st ,,
				3·00	450·0	47·97	2nd ,,
39	21	1·37	30·00	7·16	225·0	53·75	1st ,,
				2·96	529·2	52·34	2nd ,,
40	25	1·50	34·62	4·50	409·2	53·18	1st ,,
				5·00	346·2	49·99	2nd ,,
41*	20	1·61	32·13	3·36	450·0	47·15	1st ,,
				6·33	310·2	61·15	2nd ,,

TABLE I—(*Contd.*)

Number of the subject	Age in years	Surface area in m^2	B Blood urea mg. per 100 c.c.	V Vol. of urine mg. per minute	U Urine urea mg. per 100 c.c.	Maximum clearance $\frac{UV}{B}$	
42	20	1·65	26·46	3·96	281·4	42·18	1st hour
				3·73	281·4	39·69	2nd ,,
44	21	1·65	30·00	2·76	499·8	46·09	1st ,,
				3·86	428·4	55·21	2nd ,,
45	24	1·47	32·13	2·46	692·4	53·15	1st ,,
				7·16	225·0	50·19	2nd ,,
50	28	1·64	28·14	5·50	225·0	43·97	1st ,,
				2·50	475·0	42·09	2nd ,,
54	23	1·60	20·46	3·73	300·0	54·73	1st ,,
55	24	1·53	24·99	5·20	204·6	42·57	1st ,,
56	22	1·70	26·46	5·41	237·0	48·51	1st ,,
Average	22·6	1·54	27·30	3·82	352·5	45·94	..

of urine per minute for the two consecutive hours and so C_m is calculated for them (Table I); 8 of the subjects passed less than 2·0 c.c. of urine per minute for the two consecutive hours and C_s value is calculated on their behalf (Table II); C_m or C_s is calculated in the case of 12 students according as the quantity of urine passed was more than or less than 2·0 c.c. per minute during each of two hours (Table III). Six subjects could not stay for more than $1\frac{1}{2}$ hours and their C_m or C_s values calculated on the first hour specimen of urine, have been included in Table I or II. The average values for C_m and C_s are shown separately in the tables, and for striking the general average all the values have been taken into consideration.

The average C_m of 46 healthy normal Mysoreans is 46·83 c.c. The individual values range between 28·32 c.c. and 67·01 c.c. The standard deviation is 8·456. 67·01 c.c. and 28·32 c.c. are the only two figures beyond 2σ of mean value. If one σ on either side of mean is considered as Gokhale has done the range of significant variation, *viz.*,

38·38 to 55·28, covers 71·4% of the subjects. The average body surface of these subjects is 1·55 m². After correction of the body surface to 1·73 m²

TABLE II

C_s values during the two consecutive hours

Number of the subject	Age in years	Surface area in m²	B Blood urea mg. per 100 c.c.	V Vol. of urine c.c. per minute	U Urine urea mg. per 100 c.c.	Standard clearance $\frac{U\sqrt{V}}{B}$	
1*	20	1·78	24·99	1·66	600·0	30·99	1st hour
				1·75	600·0	31·77	2nd ,,
7	22	1·52	32·13	0·50	1800·0	39·61	1st ,,
				0·50	1800·0	39·61	2nd ,,
11	19	1·50	21·42	1·16	692·4	34·90	1st ,,
				1·03	750·0	35·58	2nd ,,
12	19	1·59	24·99	1·33	562·8	25·99	1st ,,
				1·26	562·8	25·34	2nd ,,
22	22	1·54	24·99	1·03	900·0	36·00	1st ,,
				1·03	900·0	36·00	2nd ,,
24	25	1·49	28·14	1·53	817·8	35·98	1st ,,
				1·03	999·6	36·07	2nd ,,
26	22	1·62	30·00	1·50	817·8	33·39	1st ,,
				1·03	1125·0	38·12	2nd ,,
51	21	1·58	32·14	0·50	1500·0	33·00	1st ,,
				0·50	1500·0	33·00	2nd ,,
52	26	1·62	30·00	1·06	1500·0	51·64	1st ,,
53	24	1·62	32·13	0·83	1125·0	31·36	1st ,,
57	25	1·80	38·13	1·30	999·6	35·47	1st ,,
Average	22·2	1·60	29·00	1·08	1029·0	34·93	

* Indicates mixed diet. Blanks indicate estimations not done.

TABLE III

C_m or C_s values during the two consecutive hours

Number of the subject	Age in years	Surface area in m^2	B Blood urea mg. per 100 c.c.	V Vol. of urine c.c. per minute	U Urine urea mg. per 100 c.c.	$C_m = \frac{UV}{B}$	$C_s = \frac{U\sqrt{V}}{B}$	
2*	19	1·77	26·46	1·00	999·6	..	37·79	1st hour
				2·08	642·6	50·58	..	2nd ,,
8	24	1·50	21·42	3·16	310·2	45·86	..	1st ,,
				1·00	817·8	..	38·18	2nd ,,
10	20	1·47	26·46	4·33	310·2	50·81	..	1st ,,
				1·00	900·0	..	34·01	2nd ,,
13	21	1·72	20·46	2·08	600·0	61·09	..	1st ,,
				0·50	1125·0	..	38·88	2nd ,,
16	25	1·64	24·99	1·83	817·8	..	44·23	1st ,,
				3·00	499·8	59·99	..	2nd ,,
23	22	1·72	28·14	2·50	600·0	53·31	..	1st ,,
				1·30	750·0	..	30·39	2nd ,,
31	22	1·55	24·99	1·40	999·6	..	47·33	1st ,,
				2·23	750·0	67·01	..	2nd ,,
43	21	1·38	30·00	1·88	900·0	..	41·16	1st ,,
				4·16	281·4	39·07	..	2nd ,,
46	19	1·42	30·00	0·83	999·6	..	30·42	1st ,,
				3·90	375·0	48·74	..	2nd ,,
47	20	1·52	26·46	1·22	817·8	..	34·08	1st ,,
				3·26	360·0	44·44	..	2nd ,,
48	25	1·64	28·14	1·16	999·6	..	38·37	1st ,,
				6·50	225·0	51·98	..	2nd ,,
49	20	1·56	26·46	0·75	..	..	..	1st ,,
				3·20	391·2	47·31	..	2nd ,,
Average	21·5	1·57	26·16	2·26	672·7	51·68	37·71	

the C_m works out to 52·26 c.c. for normal Mysoreans, which is 69% of the average value of Americans (Table IV).

TABLE IV

Comparative C_m amd C_s values

	Clearance			Standard Deviation	Surface area in m^2	Clearance calculated to a S.A. of 1·73 m^2 of subjects in		
	Mean	Maximum	Minimum			Mysore	Bombay	America
C_m ..	46·83	67·01	28·32	±8·456	1·55	52·26	44·0	75·0
C_s ..	35·95	51·64	25·34	±5·252	1·58	39·36	33·8	54·0

The average standard clearance of 23 subjects is 35·95 c.c. The individual values range between 25·34 c.c. and 51·64 c.c. The standard deviation is 5·252. 51·64 is the only figure which is beyond 2 σ of mean value. If only one σ on either side of mean is considered, the range of significant variation, *viz.*, 30·70 to 41·20 covers 76·6% of the subjects. The average body surface of these is 1·58 m^2. After correction of the body surface to 1·73 m^2 the average C_s for normal Mysoreans is 39·36 c.c. which is 73% of the American standard (Table IV). The average C_m and C_s values of Mysoreans are slightly higher than the corresponding figures for Bombay subjects. This may be really the case or it may be due to the difference in the methods employed for the estimation. But when compared with the average blood urea clearance of American subjects our figures are markedly low. Blood urea level being more or less the same, the low blood urea clearance is due to low urinary nitrogen. This is not strange to expect when one compares the protein content of the food of the two races. Low protein consumption may be the chief factor for the low urinary nitrogen and so for low urea clearance values. The estimation of protein consumption will be taken up next.

Summary

1. The average blood urea clearance of 57 normal Mysoreans between the ages of 19 and 28 years is determined adopting the technique of Moller *et al.*

2. Urea is determined by the xanthydrol micro method.

3. The average C_m is 46·83 c.c. and C_s 35·95 c.c. after correcting for the body surface to 1·73 m^2.

4. These values are low as compared with the corresponding figures for Americans but compare favourably with the figures of Gokhale obtained for Indians of the Bombay Presidency.

5. Low protein content of the diet appears to be the chief factor for the low urea clearance.

Acknowledgment

We would like to record here our thanks to the students of the University Medical College who willingly co-operated in carrying out this investigation, and to Mr. M. Narayana Iyenger of the Maharaja's College, Mysore, for his guidance in the statistical survey.

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THE EFFECT OF TEMPERATURE AND IONS ON THE IMPEDANCE OF UNSTRIATED MUSCLE AND ITS RELATION TO PERMEABILITY AND EXCITABILITY

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The experiments described previously (Singh and Mrs. Singh, 1943) have been continued with use of different technique, and the results compared with those on striated muscle. The results of electrical experiments are correlated with those of diffusion experiments.

Methods

The muscles used were frog stomach, dog stomach and frog rectus abdominis. The impedance to alternating current (1000 cycles/sec.) was measured by means of a Wheatstones bridge, the one arm of which contained the muscle and the other a variable resistance in parallel with a variable capacity. The impedance was measured according to the formula $R = \frac{r}{1 + w^2 r^2 C^2}$; in experiments on dog stomach and frog rectus abdominis this was practically equal to r. The electrodes used were of silver, one inch in diameter and of platinised platinum 8×18 mm. with the sucrose electrolyte solution.

Saline solutions were also used with silver electrodes, with or without being chlorided. The resistance of the electrodes is apt to increase with use, and so it should be checked after each observation. At least 3 to 6 readings were taken for each observation (after the muscle had been placed between the electrodes and removed therefrom) to exclude errors due to placing of the muscle. This error may amount to 20 p.c. ; otherwise the error is about 1–2 p.c. The apparatus was sensitive to 1–2 p.c.; greater sensitivity was not desired, as the experimental error amounts to as much. With salt solutions the readings could be made to 0·1 ohms and with the sucrose electrolyte solution to 1 ohm. In this paper a minimum variation of 10 ohms has only been recorded, lesser changes being neglected. In this way constant readings could be obtained for several hours.

Results

A. *With sucrose electrolyte solution*

Effect of temperature.—The effect of dog stomach using silver electrodes is shown in Table I (*a*), that on frog stomach using platinum electrodes in Table I (*b*), and that on frog rectus abdominis in Table I (*c*).

TABLE I(*a*). *Effect of temperature on the impedance of dog stomach; Silver electrodes, Ohms*

L = living muscle ; D = muscle heated to 50° C.

°C.	No. of muscle											
	1		2		3		4		5		6	
	L	D	L	D	L	D	L	D	L	D	L	D
0	4000	..	..	..	..	..	350	..	1000	..	..	..
1	3000	..	440	410	520	..	..	..	..	..	..	..
2	2000	..	..	..	..	..	330	..	900	430	360	360
3	1000	..	..	..	..	..	..	..	..	..	..	..
5	500	..	530	340	470	..	310	490	500	390	380	330
10	400	..	470	240	440	..	280	440	430	330	360	280
15	350	..	500	210	400	..	210	370	420	280	350	270
20	300	..	430	170	380	..	200	320	370	250	330	260
25	270	..	380	150	350	..	200	240	370	220	270	250
30	240	..	320	130	300	..	190	220	340	200	310	230
35	180	..	280	120	250	..	190	200	310	170	250	200
40	170	..	280	110	240	..	180	180	260	160	240	190
45	210	..	330	100	240	..	180	170	280	150	230	180
50	160	..	310	90	210	..	180	160	280	140	220	170

TABLE I(*b*). *Effect of temperature on the impedance of frog stomach; Platinum electrodes*

°C.	No. of muscle											
	1		2		3		4		5		6	
	L	D	L	D	L	D	L	D	L	D	L	D
3	..	..	..	..	510	..	320	..	260	..	260	..
5	900	..	360	918	470	350	400	460	250	..	250	..
7	1210	..	..	..	..	..	..	..	..	..	..	..
10	1000	..	380	710	530	340	360	450	270	..	270	..
15	900	960	360	650	520	330	430	440	260	..	260	..
20	810	900	590	600	510	320	330	400	270	..	270	..
25	930	860	360	560	460	310	420	350	260	..	260	..
30	740	770	290	500	520	300	420	290	260	..	260	..
35	570	..	360	..	520	300	460	280	260	..	260	..
37	560	..	..	..	..	..	..	..	..	..	..	..
40	590	700	250	480	530	290	430	270	260	..	260	..
45	550	660	290	440	500	280	420	260	260	..	260	..
50	350	650	220	420	500	270	430	250	290	..	290	..

TABLE I(*c*). *Effect of temperature on impedance of frog rectus abdominis; Silver electrodes*

°C.	No. of muscle											
	1		2		3		4		5		6	
	L	D	L	D	L	D	L	D	L	D	L	D
− 2	3710	..	..	..	..		..	..	..	..	..	..
− 1	3000	..	..	..	..	..	..	..	..	..	..	..
0	500	..	..	..	..	..	..	..	..	..	640	..
1	..	..	..	..	..	..	..	..	..	..	..	..
2	380	..	..	..	..	..	590	280	700	..	740	..
3	..	..	..	..	550	..	..	..	..	..	..	..
4	..	..	860	..	..	..	..	..	..	..	..	..
5	490	..	830	290	580	330	690	270	690	170	740	310
10	350	..	720	290	490	300	590	260	600	160	460	230
15	330	..	690	270	450	300	420	240	660	150	460	220
20	330	..	550	250	400	290	400	190	470	140	370	210
25	450	..	500	220	370	270	380	180	520	130	380	200
30	310	..	510	200	400	260	360	170	660	120	370	190
35	290	..	420	180	340	240	330	160	410	110	370	150
40	260	..	410	150	370	220	330	150	520	100	370	140
45	190	..	240	120	190	180	180	140	200	80	340	130
50	100	..	130	110	130	130	140	130	120	70	130	320

The results are as described previously. The effect of very low temperatures about 0° C., is to greatly increase the impedance; the permeability is probably greatly decreased. This may account for the fact that frog heart beats in the absence of calcium in cold (Heilbrunn, 1937), lowering of temperature and cold, both decreasing permeability. But from the tables and from the previous paper, it is seen that cold may both increase or decrease the permeability.

One difference was noted between these and previous results, that the resistance of dead muscle may be much lower than that of living muscle at all temperatures, though in others it is actually higher, especially at low temperatures. The resistance of dead muscle would depend upon its electrolyte content; if that diffuses out then the resistance of dead muscle would be higher than that of the living muscle. Further the previous muscles were more resistant, 70° C. being required to lower the resistance to the same extent as 50° C. in these muscles.

Another feature in these experiments is that at high temperatures, the resistance may actually increase at 40° C. in striated and 45° C. in unstriated muscle. In striated muscle there is rapid drop after 45° C. The resistance of unstriated muscle appears to increase prior to death.

These results are in agreement with the physiological reactions of unstriated muscle, which shows heat paralysis, increase in adaptation, decrease of viscosity at high temperatures, all being probably due to liberation of calcium. Liberation of calcium from protoplasm at high temperatures has been shown by Mazia and Clark (1936).

B. *With saline solutions*

The method is not very sensitive and only large variations can be recorded.

Unstriated and striated muscles.—The resistance of unstriated muscle is lower than that of striated muscle, that of frog rectus abdominis being about 50 ohms, and that of frog stomach of equal thickness and area, about 10 ohms; that of dog stomach is about 20 ohms, while that of dog abdominal striated muscles about 30–40 ohms. This is in agreemet with the fact that diffusion in plain muscle is easier than in striated muscle.

Thus when *Mytilus* unstriated muscle is immersed in a hypotonic solution of sodium chloride it swells and gains base. This swelling is prevented if the concentration of calcium is increased (Singh, 1938*a*); the muscle then behaves like an osmotic cell and loses weight in hypertonic saline. Calcium (0·01–0·02 M $CaCl_2$) thus decreases the permeability to sodium chloride. As frog striated muscle does not swell in hypertonic sodium chloride solution its membrane is less permeable than that of striated muscle. These results are in agreement with the fact that striated muscle is more excitable to electric current and less to chemical stimulation than plain muscle.

Effect of compression.—The resistance depends upon the pressure to which the muscle is subjected. If the compression is increased the resistance increases, even though the electrodes come closer.

This is due to squeezing out of the saline solution out of the inter-fibre spaces, and is comparable to squeezing out of base in *Mytilus* muscle (Singh, 1939).

The above experiment shows that any slight increase of resistance during contraction whether isotonic or isometric is of doubtful significance, as the inter-fibre space is bound to diminish ; a decrease is of greater significance.

Effect of temperature.—The fluctuations seen with the sucrose electrolyte solution are not observed; there is a continuous decrease of resistance with temperature, about 2 per cent. per degree Centigrade. During the experiments, the temperature should be noted.

Effect of death.—The resistance falls greatly if the muscle is heated to 50° C. or killed otherwise. During the course of the day, the resistance falls by about 20 p.c., in 6 hours, and the various substances become less effective in causing an increase of resistance; a decrease may be produced. In plain muscle, the resistance of which is about 50 p.c. that of striated muscle, the substances that produce increase in resistance of the latter hardly produce any effect. The resistance of both muscles keeps better in acid solutions (pH 6·5–6·2). The resistance of striated muscle falls as it passes into rigour after some hours.

Effect of monovalent cations: Effect of hydrogen ions.—Hydrogen ions increase the resistance of both striated and unstriated muscles (Table II *a*); they decrease the gain of base by *Mytilus* muscle (Table II *b*), the permea-

TABLE II(*a*). *Effect of hydrogen ions on impedance of dog stomach and frog rectus; ohms. Immersion for 30 min.*

pH	Dog stomach							Frog rectus					
	1	2	3	4	5	6	7	1	2	3	4	5	6
6·5	40	40	40	40	20	20	20	50	50	60	50	50	50
9	20	20	20	20	20	20	30	30	40	40	20	20	20
8·7	20	20	20	20	20	20	20	30	40	40	20	20	20
8·3	20	20	20	20	20	20	20	40	40	40	20	20	20
7·8	30	30	30	30	20	20	20	40	40	40	20	20	20
7·4	30	30	30	30	20	20	20	40	30	30	20	20	20
7	30	30	30	30	20	20	20	40	30	30	30	30	30
6·5	40	40	40	40	20	20	20	30	30	30	40	40	40
6·2	40	40	40	40	20	20	20	30	30	30	40	40	40
6	40	40	40	40	20	20	20	30	30	30	40	40	40
5·2	40	30	40	30	20	20	20	30	30	30	50	50	50
5	40	30	40	30	20	20	20	30	30	30	50	40	40
4	..	..	..	..	..	..	..	30	30	30	40	40	40
2·5	..	..	..	..	..	..	..	30	30	20	30	30	20
1·03	10	10	10	10	10	10	10	10	10	10	10	10	10

TABLE II(*b*). *Effect of hydrogen ions on the base content of* Mytilus *muscle, soaked in isotonic sodium chloride for 16 hours*
Base in m. eq. per gram of wet muscle before soaking

pH	No. of muscle					
	1	2	3	4	5	6
7·8	0·532	0·602	0·469	0·503	0·559	0·685
5·6	0·370	0·425	0·378	..	..	..
4·6	..	..	..	0·363	0·421	0·370

bility of erythrocytes (Blinks, 1942), the diffusion of potassium and ammonium in unstriated muscle (Gokhale and Singh) and in striated muscle (Fenn and Cobb, 1934).

Hydrogen ions to a certain extent, diminish the excitability to chemical stimulation and increase that to electric current; they cause inhibition. Large concentrations may have opposite effect.

Effect of potassium.—Small concentrations (less than 0·02 M KCl) have no significant action on frog rectus or dog stomach. Larger concentrations increase the resistance of frog rectus (Table III *a, b*). Very large concentrations 0·5 M KCl, isotonic with sea water, markedly decrease the resistance of both, as much as is produced by killing the muscle with heat. The damage produced may be permanent, or the muscle may take 2–3 hours to recover. A decrease in the resistance of 3 frog recti by small concentrations of potassium, has been observed. The increase in the resistance of the muscle is a very constant phenomenon, and is still produced if the muscle is previously kept in calcium-free saline for 3–4 hours; it is associated with contracture. The increase in resistance lasts several hours (Fig. 1) after

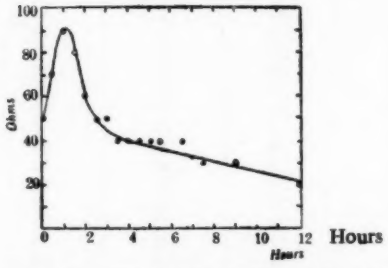

FIG. 1. Frog rectus. Effect of 0·1 M KCl on impedance

which the resistance declines; it is however difficult to say whether this decline is produced by potassium, or it is due to deterioration of the muscle, produced by time. Duboisson (1937) found increase of resistance during contracture; Boyle and Conway (1941) found that potassium diminished the permeability to sodium.

Potassium is known to increase the permeability though small concentrations definitely diminish the permeability of *Mytilus* muscle to sodium and barium (Table III *c, d*). The increase of resistance in frog rectus may be due to liberation of calcium.

Large concentrations of potassium destroy the normal permeability, as is also shown by the fact that in *Mytilus* muscle, the concentration of

TABLE III(*a*). *Effect of potassium on the impedance of dog stomach and frog rectus; immersion for* 30 *min.*

Solution	Dog stomach						Frog rectus					
	1	2	3	4	5	6	1	2	3	4	5	6
Normal	20	20	20	20	20	20	50	50	50	50	50	50
Normal	20	20	20	20	20	20	50	50	50	50	50	50
Normal	20	20	20	20	20	20	50	50	50	50	50	50
0·02 M KCl	20	20	30	20	20	20	60	60	60	70	70	60
0·1 M KCl	20	20	30	20	20	20	90	90	100	110	100	90
0·5 M KCl	10	10	10	10	10	10	10	10	10	10	10	10
Heat 50° C.	10	10	10	10	10	10	10	10	10	10	10	10

TABLE III(*b*). *Effect of potassium* (0·1M KCl) *on impedance of dog stomach and frog rectus; time action relations*

Hours	Dog stomach			Frog rectus		
	1	2	3	1	2	3
0	20	20	20	50	50	50
½	20	20	20	70	70	80
1	20	20	20	90	90	90
1½	20	20	20	80	80	90
2	20	20	20	60	70	90
2½	20	20	20	50	60	70
3	20	20	20	50	60	70
3½	20	20	20	40	40	60
4½	20	20	20	40	60	60
5½	20	20	20	40	50	60
6½	20	20	20	40	40	50
7½	20	20	20	30	30	30
9	20	20	20	30	30	30
12	20	10	20	20	20	20

TABLE III(*c*). *Effect of* 0·01 M KCl *on the permeability of* Mytilus *muscle to sodium. Muscle pairs immersed in* 0·564 M NaCl *for* 16 *hours; figures show gain of base in m.eq. by gram of wet muscle. One muscle immersed in control solution, the other in the experimental solution*

No. of muscle	In presence of potassium	In absence of potassium	Percentage increase in absence of potassium
1	0·329	0·441	34
2	0·379	0·406	8
3	0·349	0·468	31
4	0·375	0·450	20
5	0·386	0·452	17
6	0·570	0·729	28

TABLE III(*d*). *Effect of* 0·01 M KCl *on the permeability of* Mytilus *muscle to barium* (0·37 M $BaCl_2$)

No. of muscle	In presence of potassium	In absence of potassium	Percentage increase in absence of potassium
1	0·343	0·432	26
2	0·588	0·648	10
3	0·364	0·437	20
4	0·344	0·476	40
5	0·424	0·547	29
6	0·550	0·687	24

TABLE III(*e*). *Effect of* 0·5 M KCl *on the base content and concentration of base in* Mytilus *muscle* Ca = 0·01 M $CaCl_2$

No of muscle	Ca		−Ca		Percentage excess in absence of Ca
	Gain of base	Concentration	Gain of base	Concentration	
1	0·774	0·437	0·96	0·46	24
2	0·545	0·306	1·18	0·45	115
3	0·749	0·420	1·12	0·44	47
4	0·660	0·430	0·96	0·48	45
5	0·604	0·430	0·92	0·41	52
6	0·858	0·440	1·13	0·47	31
7	..	0·386	..	..	..
8	..	0·350	..	..	..
9	..	0·387	..	..	..
10	..	0·384	..	..	..
11	..	0·371	..	..	..
12	..	0·386	..	..	..
13	0·745	..	1·216	..	63
14	0·988	..	1·456	..	47
15	0·690	..	1·138	..	67
16	0·990	..	1·412	..	42
17	0·730	..	1·133	..	55
18	0·965	..	1·455	..	50

base becomes equal to that of *Mytilus* saline (Table III *e*). Calcium has a protective action.

Effect of ammonium.—Ammonium acts like potassium, but it is less powerful. It increases the resistance and produces contracture (Table IV).

TABLE IV. *Effect of ammonium on the impedance of dog stomach and frog rectus*

Solution	Dog stomach									Frog rectus								
	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
Normal	20	20	20	70	80	90	40	60	40	50	50	50	60	60	50	40	50	50
0·1 M NH_4Cl	20	20	20	50	70	80	50	70	50	60	60	60	60	60	40	50	60	60
0·1 M NH_4SCN	..	..	..	..	..	..	..	..	..	100	100	100	120	110	90	100	90	120
0·56 M NH_4Cl	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Effect of sodium.—Sodium chloride may increase or decrease the resistance of frog rectus; it decreases the resistance of dog stomach sometimes (Table V).

TABLE V. *Effect of sodium chloride on impedance of dog stomach and frog rectus*

Solution	Dog stomach						Frog rectus					
	1	2	3	4	5	6	1	2	3	4	5	6
Normal	20	20	20	30	30	30	50	60	40	40	50	50
NaCl	20	20	20	20	20	20	40	40	40	40	40	40
Normal	20	20	20	30	30	30	60	60	50	40	60	60
0·56 M NaCl ..	10	10	10	10	10	10	10	10	10	10	10	10

Effect of lithium.—No definite effect has been noticed on dog stomach; large concentrations isotonic with sea-water greatly diminish the resistance. In frog rectus the resistance may increase or decrease (Table VI). It appears that the monovalent cations may increase or decrease the resistance in the order $Li < Na < NH_4 < K$ or $Na < Li < NH_4 < K$.

TABLE VI. *Effect of lithium saline on impedance of frog rectus*

Solution	No. of muscle											
	1	2	3	4	5	6	7	8	9	10	11	12
Normal	50	60	50	60	50	50	50	50	50	50	50	50
LiCl	40	40	40	40	40	50	70	70	70	70	70	70
Normal	50	50	40	40	50	50	50	50	50	50	40	40
0·56 M LiCl	10	10	10	10	10	10	10	10	10	10	10	10

Effect of divalent cations: Effect of calcium.—Some of the effects of calcium are shown in Table V, the difference between the effects of normal solution and sodium chloride being due to the calcium. Calcium (0·00824 M $CaCl_2$) however has a dual effect, the resistance increasing in its absence (Table VII *a*). The increase in resistance in its absence is associated with contracture. It may actually be due to liberation of calcium; calcium lack may liberate calcium (Heilbrunn, 1937). Large concentrations decrease the resistance. Calcium decreases the permeability of *Mytilus* muscle to sodium and barium (Table VII *c, d*; Singh, 1938) and decreases the loss of potassium from frog stomach (Gokhale and Singh).

TABLE VII (*a*). *Effect of Ca-free saline on impedance of frog rectus*

Solution	pH 5·2			pH 8·5		
	1	2	3	4	5	6
Normal	40	50	50	40	30	40
Ca-frog saline	60	60	70	70	60	70
Normal	40	50	40	40	40	40

TABLE VII(*b*). *Effect of calcium on impedance of dog stomach and frog rectus*

	Dog stomach			Frog rectus		
	1	2	3	1	2	3
Normal	20	20	20	40	50	50
0·02 M $CaCl_2$	20	20	20	50	60	60
0·1 M $CaCl_2$	20	20	20	60	70	70
0·3 M $CaCl_2$	10	10	10	10	10	10

Table VII(*c*). *Effect of calcium* (0·01 M $CaCl_2$) *on the permeability of* Mytilus *muscle to sodium chloride*

No. of muscle	Ca	− Ca	Percentage increase in absence of Ca
1	0·312	0·697	123
2	0·286	0·876	209
3	0·290	0·628	118
4	0·310	0·722	133
5	0·295	0·450	59
6	0·351	0·791	126
7	0·345	0·548	58
8	0·323	0·392	18
9	0·306	0·447	46
10	0·438	0·476	8
11	0·343	0·516	50
12	0·348	0·588	68

TABLE VII(*d*). *Effect of calcium on permeability to barium* (0·37 M $BaCl_2$)

No. of muscle	Ca	− Ca	Percentage increase in base in absence of calcium
1	0·431	0·451	4
2	0·343	0·482	10
3	0·373	0·440	7

Effect of magnesium.—This had no significant effect on dog stomach; 0·08 M $MgCl_2$ increased the resistance of frog rectus (Table VIII *a*). Large concentrations (0·3 M) decrease the resistance of both. In *Mytilus* muscle small concentrations of magnesium diminish the permeability to sodium and potassium (Table VIII *b*, *c*).

TABLE VIII(*a*). *Effect of magnesium on the impedance on dog stomach and frog rectus*

Solution	Dog stomach			Frog rectus					
	1	2	3	1	2	3	4	5	6
Normal	20	20	20	50	50	50	50	50	50
0·02 M M_gCl_2	20	20	20	50	50	50	50	50	50
0·04 M M_gd	20	20	20	50	50	50	50	50	50
0·06 M MCl_2	20	20	20	50	50	60	60	50	50
0·08 M My	20	20	20	60	70	70	70	70	60
0· 3 M MyCl	10	10	10	10	10	10	10	10	10
Normal	20	20	20	20	20	20	20	20	20

TABLE VIII(*b*). *Effect of magnesium* (0·07 M M_gCl_2) *on the permeability of* Mytilus *muscle to sodium* (0·56 M)

No. of muscle	Mg	– Mg	Percentage of gain of base in absence of magnesium
1	0·366	0·452	23
2	0·445	0·536	20
3	0·465	0·679	46
4	0·411	0·582	41
5	0·365	0·463	24
6	0·398	0·547	37

TABLE VIII(*c*). *Effect of magnesium* (0·07 MgCl) *on the permeability of* Mytilus *muscle to potassium* (0·56 M)

No. of muscle	Mg	– Mg	Percentage of gain of base in absence of magnesium
1	0·682	0·735	8
2	0·564	0·649	15
3	0·680	0·770	13

Effect of barium and strontium.—0·02 M $BaCl_2$ had no significant effect on dog stomach, but increased or decreased the resistance of frog rectus (Table IX). Strontium (0·02 M $SrCl_2$) had no significant action.

TABLE IX. *Effect of barium* (0·02 M $BaCl_2$) *impedance of dog stomach and frog rectus*

Solution	Dog stomach			Frog rectus								
	1	2	3	1	2	3	4	5	6	7	8	9
Normal	20	20	20	40	50	50	40	50	50	50	40	40
0·02 M $BaCl_2$	20	20	20	50	60	60	60	30	30	30	30	40
Normal	20	20	20	40	50	50	40	50	50	50	40	40

Effect of anions.—Like other agents, the anions have a dual action. They increase the resistance of frog rectus in the order $Cl < Br < NO_3 < I < SCN$, if they cause contraction; otherwise they decrease the resistance of both dog stomach as well as frog rectus (Table X). If they increase the resistance, then when they are withdrawn, there is a marked fall of resistance, which may be related to withdrawal contractions. Like potassium,

TABLE X(*a*). *Effect of anions on the impedance of dog stomach*

No. of muscle	Cl	Br	NO_3	I	SCN	CN
1	70	50	50	30	30	20
2	60	40	40	30	20	20
3	60	30	30	30	20	20
4	50	40	30	30	20	20
5	40	30	30	20	20	20
6	50	40	30	30	30	20
7	20	20	20	20	20	20
8	20	20	20	20	20	20
9	20	20	20	20	20	20

TABLE X(*b*). *Effect of bromide on impedance of frog rectus*

No. of muscle	1	2	3	4	5	6
Normal	30	40	40	50	50	50
Bromide saline	50	60	60	40	40	40
Normal	30	40	40	50	50	40

TABLE X(*c*). *Effect of nitrate on impedance of frog rectus*

No. of muscle	1	2	3	4	5	6
Normal	30	40	50	50	50	50
Nitrate	60	60	60	50	40	40
Normal	30	40	40	50	50	50

TABLE X(*d*). *Effect of iodide on impedance of frog rectus*

No. of muscle	1	2	3	4	5	6
Normal	50	50	50	50	50	50
Iodide	70	70	70	70	60	60
Normal	40	20	20	20	30	40

TABLE X(*e*). *Effect of thiocyanate on impedance of frog rectus*

Solution	1	2	3	4	5	6
Normal	40	40	50	60	50	50
SCN	80	90	90	40	40	40
Normal	30	30	50	50	50	50

TABLE X(*f*). *Effect of anions on the permeability of* Mytilus *muscle to sodium (gain of base)*

No. of muscle	PAIRS							
	A		B		C		D	
	NaCl	NaBr	NaBr	$NaNO_3$	$NaNO_3$	NaI	NaI	Na SCN
1	0·435	0·562	0·586	0·570	0·625	0·794	0·475	0·525
2	0·379	0·463	0·423	0·570	0·687	0·800	0·758	0·632
3	0·400	0·519	0·523	0·522	0·606	0·766	0·870	0·888
4	0·562	0·594	0·531	0·763	0·753	0·795	0·946	0·775
5	0·442	0·470	0·509	0·690	0·474	0·608	0·700	0·543
6	0·400	0·517	0·538	0·788	0·392	1·168	0·706	0·880

cyanide destroys the permeability of *Mytilus* muscle. Thiocyanate increases the loss of potassium from frog stomach (Gokhale and Singh).

Effect of drugs—Drugs, though they have such marked physiological action, were without any marked effect on the resistance, comparable to those of the ions; their actions are doubtful.

Effect of adrenaline.—On dog stomach there was no significant action. This is due to the low resistance of plain muscle. In frog rectus when the resistance becomes low, substances are ineffective in causing an increase. Out of 8 frog recti, in 2 muscles 1 in 10^6, slightly decreased the resistance (20 p. c.), in 3 muscles there was no significant action, and in 3 muscles 1 in 10^5 adrenaline, decreased the resistance. As however, this effect was not reversible, it is difficult to say whether it was significant.

In sucrose electrolyte solution of normal or half osmotic pressure, adrenaline had no effect on dog stomach or frog rectus.

Effect of ephedrine.—On dog stomach there was no significant action. Out of 6 frog recti, 1 in 10^5 ephedrine reduced the resistance slightly (20 p.c.) in 4 muscles. In *Mytilus* muscle, there was no effect on gain of base.

Effect of caffeine.—On dog stomach there was no significant action. In 6 frog recti, 1 in 100 approximately, caused a marked contraction, yet there was no increase, but a 40 p.c. decrease of resistance. As the action was irreversible, it is difficult to say whether it was significant. In 3 *Mytilus* pairs, however, the base content of 3 untreated muscles soaked for 16 hours was 0·263, 0·224, 0·275 m.eq./g.; in caffeine treated muscles, the base content was 0·260, 0·471, 0·354 m.eq., so that there appears to be an increase of permeability.

Effect of eserine.—1 in 10^5 to 10^4, slightly increased the resistance of 6 frog recti; there was no effect on dog stomach. This concentration of eserine diminishes tone in *Mytilus* muscle, and increases the excitability to alternating current in dog stomach and *Mytilus* muscle.

Effect of acetylcholine (1 in 10^4).—In 4 out of 6 eserinised recti, there was a slight increase (20 p.c.) in impedance during contracture.

Effect of veratrine.—1 in 1,000 slightly decreased the impedance (20 p.c.) in 4 out of 6 recti. It had no effect on the base or sodium content of *Mytilus* muscle.

Effect of cocaine.—This had no significant action, though in 3 out of 6 recti, 1 in 1000, produced a slight decrease of impedance (20 p.c.). Strychnine (1 in 100) had a similar effect.

In *Mytilus* muscle large concentrations of the above drugs increase the excitability to potassium, and decrease that to electric current. But as the effect on impedance was not reversible, it is difficult to say, whether the increase in excitability to potassium can be correlated with the decrease of impedance. In frog stomach they produce inexcitability.

Discussion

During stimulation of plain muscle with alternating current, tone may be diminished; similarly when stimulated with acetylcholine or potassium tone is found to be decreased just after stimulation. This suggests, that, during contraction produced by these substances, tone decreases; the permeability is, therefore, decreased. This is in agreement with the findings above, that during contracture, there is an increase of resistance, and also

with those of Fenn, Noonan and Haege (1941), that there is no increase in the permeability to radio-active potassium during stimulation of frog muscle.

During stimulation with alternating current. the sensitivity of *Mytilus* muscle to potassium may decrease or increase, the latter action suggesting an increase in permeability. Similarly when frog stomach is stimulated with ammonium in the presence of thiocyanate, the rapid decline of tension is recovered from, suggesting a decrease of permeability followed by an increase. This is in agreement with the fact that resistance may also decrease during contraction.

Ions have a dual effect on excitability in that they may either cause inhibition or excitation; with this is correlated the fact that they either increase or decrease the resistance, or decrease or increase the permeability. It is possible that the decrease of permeability by some of these ions is an indirect effect, probably by liberation of calcium; with this is correlated the fact that calcium may reverse an excitatory into an inhibitory effect.

The permeability of *Mytilus* muscle to ions varies the order $Li < Na < NH_4 < K$ and $Cl < Br < NO_3 < I < SCN$. Their inhibitory and excitatory effects also vary in the same order; similarly their effect on resistance or permeability. It thus appears that both excitation and inhibition are associated with a common property of these ions, which is probably their power to enter the muscle membrane. Excitation or inhibition thus appears to be produced when the ions enter the muscle membrane. If inhibition is dependent upon the liberation of calcium by the ion then the more of it enters, the greater the inhibitory effect, and if the liberation of calcium does not occur then greater will be its excitatory effect. Thus the ion to which the muscle is more permeable is also more inhibitory or excitatory than the ion to which the muscle is less permeable. Thus increase in permeability will favour both inhibition as well as excitation by ions; this is in agreement with the fact that same factors that facilitate excitation may also facilitate inhibition (Singh, 1942).

Ions may however produce inhibition directly by having a calcium-like action, instead of through liberation of calcium; thus the complicated nature of inhibition is apparent (Singh, 1944). In the former case the phase of decreased permeability, should be preceded by a transient opposite phase. Thus Duboisson (1937) found a rapid decrease of impedance followed by a decrease or increase in frog muscle. The latter differences can be explained that increase of impedance will occur if calcium is liberated, and a decrease if it is liberated in insufficient quantity, so that probably more calcium is liberated during isometric contraction than during isotonic. This is in

agreement with the fact that stretching increases adaptation in plain muscle, so that probably more calcium is liberated (Singh, 1944).

The action of ion that liberates calcium may be antagonistic to that of calcium, so that inhibition is followed by contraction (Singh, 1944). It must be remembered that, in this and previous papers, effects which have been ascribed to liberation of calcium may be due to some other substance; the suggestion is made, owing to the fact that calcium has a similar action, and some of the effects do not occur in the absence of calcium.

If excitation is assumed to be due to entrance of ions into the muscle membrane, or an outer zone, then inhibition would be associated with an opposite change, mere change in permeability not being sufficient to account for the reversal; calcium, thus, though preventing the ingress of ions, would favour their egress, conferring rectifying properties on the muscle membrane. There is some experimental support for this. In the absence of calcium, *Mytilus* muscle gains water and base, and this is reversed in its presence—syneresis. The entrance of ions is not merely determined by permeability changes, but also by colloidal affinity. Potassium and other cations and anions in small concentrations, will have a calcium-like effect, but an opposite action in large concentrations.

The liberation of calcium is associated with adaptation, and is probably the invariable result of excitation.

Large concentrations of ions greatly decrease the resistance. The loss of excitability is then due to loss of normal permeability, the muscle becoming inexcitable if the permeability is too much increased. Though death is associated with increase in permeability, an opposite change may also be produced, especially by acids or heat. This is shown by change in resistance and also by diffusion; in pH 4·4, *Mytilus* muscle probably dies if soaked for 16 hours, but the base content may not become equal to that of sea-water.

The method used herein is very simple compared to those of other workers (Cole and Curtiss, 1937), but still the information is useful, as there is a close correspondence with diffusion experiments.

Summary

1. Generally the resistance of striated and unstriated muscle decreases with temperature; there may be increase at high temperatures or before death. At about 0° C., the resistance greatly increases.

2. Compression increases the resistance, owing to squeezing out of fluid from between the fibres; this makes any slight increase in the resistance of muscle during contraction of doubtful significance.

3. The resistance of plain muscle is less than that of striated muscle, and the former is more permeable than the latter.

4. Hydrogen ions increase the resistance of striated as well as plain muscle; they diminish the permeability of plain muscle and cause inhibition.

5. Cations increase or decrease the resistance of striated muscle in the order $Li < Na < NH_4 < K$.

6. Anions decrease the resistance of plain as well as striated muscles in the order $Cl < Br < NO_3 < I < SCN$; they increase the permeability of plain muscle in the same order. In skeletal muscle they increase the resistance in the same order if contracture occurs.

7. Calcium increases the resistance of plain as well as striated muscles; in the latter it may have an opposite action. In plain muscle it decreases permeability to ions.

8. Magnesium increases the resistance of striated muscle; barium may increase or decrease, while strontium has no significant action.

9. Large concentrations of ions greatly decrease the resistance.

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DISEASES OF PAN *(PIPER BETLE)* IN SYLHET, ASSAM

Part I. The Problem and its Economic Importance

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I. Introduction

Pan (*Piper betle*, Lin.) is cultivated extensively in Sylhet by a special class of people known as the *baruis* with whom it is a hereditary profession. *Pan* vines are grown in specially constructed houses called *borojes* whose sides and roof are usually made of grass or straw on a light framework constructed with bamboo. The vines are grown in rows on ridges and tied to bamboo strip supports. The *boroj* may be a few square yards or extend up to as much as a quarter of an acre and several *borojes* are often placed side by side covering a large area. These *borojes* are mostly located on the banks of the more important rivers, *viz.*, Surma, Kusiara, Manu, Dholai, Khowai, Kachua and Juri. The method of cultivation has been described in detail by the author (1939).

II. Historical

Foot-rot diseases of *pan* have been reported from most of the major betel-vine growing areas. Reports of these diseases have been made by Dastur (1926) from the Central Provinces, by Sundararaman (1928) from the Madras Presidency, by U Thet Su (1929) from Upper and Lower Burma, by Uppal (1931) from the Bombay Presidency and by McRae (1934) from Bengal. Thompson (1929) reported it from Malaya and stated that the damage done was considerable.

In Sylhet serious diseases of *pan* threatening its cultivation were first reported in 1929. Investigations into the problem by the Mycological Assistant, Assam, led to the identification of a wilt disease due to *Rhizoctonia solani* Kuhn though it will be seen later that this is not the most important disease. Methods of control consisting of the spraying of Bordeaux mixture was recommended but it was not found effective and did not gain favour with the *pan* growers. The severity of the diseases was increasing every year and the Government was urged to make a thorough study of the problem. Systematic scientific study of the problem was therefore begun in 1939 with

the appointment of a special staff for the purpose. A survey was first made to assess the loss and the gravity of the problem and investigations into the causes of the diseases and the methods of their control and prevention thereafter begun. In the present and subsequent papers the results of these studies will be presented

III. Economic Importance

A very careful survey was made by visiting all the *pan*-growing tracts. A very large number of the *pan* growers were examined; the acreage under *pan* before and after the prevalence of the *pan* diseases was ascertained by questioning the *pan* growers; the number of death of plants due to diseases was estimated by actual counting and the percentage of death calculated therefrom. The survey has brought out the following facts:

(i) *Decrease in acreage.*—Due to the prevalence of the *pan* diseases it has been found that there has been a considerable decrease in the acreage under *pan*. The acreage was estimated by questioning the growers of each village. The data are presented in Table I.

TABLE I

Decrease in acreage under pan *due to the prevalence of the* pan *diseases*

Subdivision	Area under *pan*		Decrease in acreage due to *pan* diseases
	1929-30	1939-40	
North Sylhet	14·5	7·6	6·9
South Sylhet	563·0	226·0	337·0
Habiganj	578·5	353·9	224·6
Karimganj	357·8	92·3	265·5
Sunamganj	..	..	..
TOTAL ..	1513·8	679·8	834·0

On account of the diseases of *pan* its cultivation has become to a very great extent unporfitable and so many of the growers have decreased the area under *pan* and quite a large number have given up its cultivation altogether.

(ii) *Decrease in the number of families growing* pan.—It has also been brought out by the survey that a large number of the *baruis* have entirely given up the cultivation of *pan* finding it unprofitable. The total number of families engaged in *pan* cultivation before and after the prevalence of the *pan* diseases are presented in Table II.

TABLE II

Decrease in the number of families growing pan *due to the prevalence of the* pan *diseases*

Subdivision	Number of families engaged in *pan* growing		Decrease in the number of families growing *pan*
	1929-30	1939-40	
North Sylhet	87	42	45
South Sylhet	1899	1572	327
Habiganj	1787	1362	425
Karimganj	936	372	564
Sunamganj	..	..	..
TOTAL ..	4709	3348	1361

(iii) *Life of a boroj.*—It has been found during the survey that before the prevalence of the *pan* diseases a *boroj,* when once raised used to last and continue giving crops for 15–30 years; some of the *borojes* lasted even for more than 50 years. But on account of the *pan* diseases it has been found that a *boroj* lasts on an average for 3–5 years after which period it becomes necessary to destroy the old one and to raise a new one. This means an extra expenditure and a *barui* is required to spend Rs. 600 to Rs. 700 every time he raises a new *boroj* for every acre of land. On the contrary he can keep an old *boroj* in good condition by making annual repairs which cost on an average Rs. 300 for every acre of land. This means considerable monetary loss to the growers.

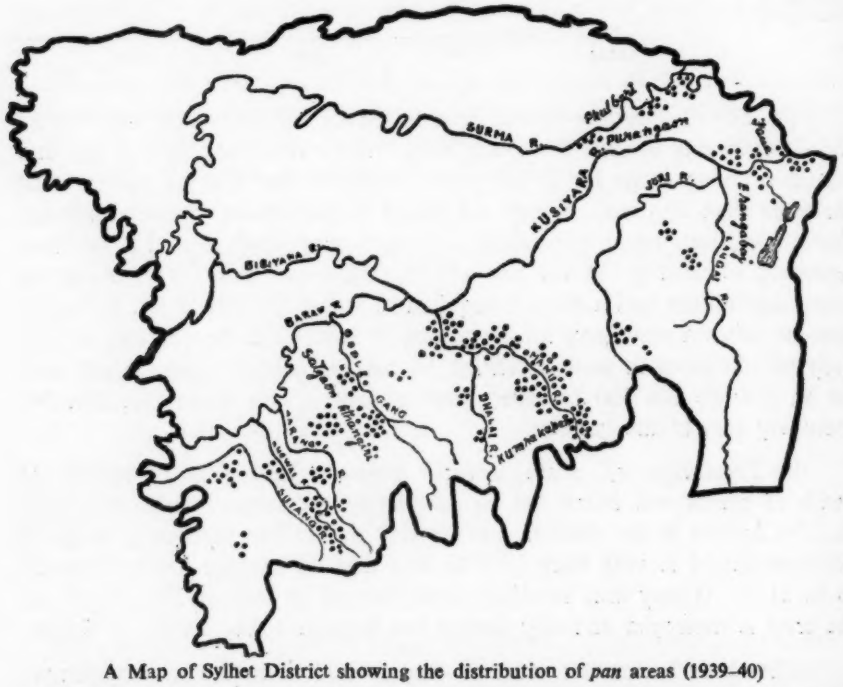
(iv) *Percentage of death and the monetary loss.*—The percentage of death of plants was found out by calculating the actual number of deaths in 2798 *borojes* in the different *pan*-growing tracts. The percentage of death has been found to vary from 17·7 to 97·8 and the average has been found to be 51·7. It may thus be safely stated that on an average 50 per cent. of the crop is destroyed annually due to *pan* diseases in the district of Sylhet.

It has been found that when no damage is done to the crop by fungus diseases on an average 80 lacs leaves are obtained from one acre of land per annum. Taking 3000 leaves per rupee as the average price of *pan* the annual gross income may be estimated at Rs. 2,500 per acre. Now it has been found that on an average 50 per cent. of the crop is destroyed annually by *pan* diseases; the monetary loss would therefore come to be Rs. 1,250 per acre per year. The total monetary loss for the district is presented in Table III.

TABLE III

Yearly monetary loss due to pan *diseases*

Subdivision	Acreage under *pan* at present	Rate of loss per acre	Total yearly monetary loss
		Rs.	Rs.
North Sylhet	7·6	1,250	9,500
South Sylhet	226·0	,,	2,82,500
Habiganj	353·9	,,	4,42,373
Karimganj	92·3	,,	1,15,375
TOTAL ..	679·8		8,49,748



A Map of Sylhet District showing the distribution of *pan* areas (1939–40)

It will be found from the data presented in Table III that the *pan* growers of Sylhet suffer annually a monetary loss of Rs. 8,49,748 due to the prevalence of the *pan* diseases.

IV. Diseases

The most important fungus diseases of *pan* in the district are the foot-root and leaf-rot diseases. Both of them have been found to be caused by the fungus *Phytophthora parasitica* Dastur. They are of very general occurrence over

all the *pan*-growing tracts except the Satgaon-Athangiri tract where they are completely absent. They are most serious in certain areas where they have caused the most severe damage and have completely wiped out many of the *pan borojes*; in other places they have been found slightly less in their severity but still doing considerable damage and heavy losses every year.

The next important disease is the *Rhizoctonia* root-rot. This has been found to be serious only in the villages Amtail, Bhowanipur and Hariram-pur on the Juri bank; Baraigram, Inainagar, Ahmedpur, Bagmara, Kutuk-nagar, Pakhiala round about the Barlekha Railway Station and in the villages Solan, Ganderghar, Tajpur, Sultanpur, Daudpur, Ranchap and Gazipur on the Manu bank.

The third disease in order of importance is a *Sclerotial* wilt. But this has been observed only in the villages Kumrakapan, Purangaon, Phulbari, Faua and Erangabaj and absent in the other localities. The damage done is however considerable.

The fourth disease is a leaf spot due to *Gloesporium* sp. This has been found to occur all over the *pan*-growing tracts. But the damage done has been found to be negligible.

V. Summary

Pan diseases have been found to be a serious menace in the district of Sylhet. They were first reported in 1929.

A careful survey has revealed that due to the *pan* diseases the area under *pan* in the district has decreased from 1,513·8 to 679·8 acres; the number of families growing *pan* has decreased from 4,709 to 3,348; the life of a *boroj* has decreased from 15–30 years to 3–5 years and the yearly monetary loss due to the death of the plants comes to Rs. 8,49,748 !

The diseases of *pan* according to their relative importance have been found to be the *Phytophthora* foot-rot and leaf-rot, the *Rhizoctonia* root-rot, the *Sclerotial* wilt and a *Gloesporium* leaf spot.

Acknowledgement

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DISEASES OF PAN (*PIPER BETLE*) IN SYLHET, ASSAM

Part II. Phytophthora Foot-rot and Leaf-rot

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(With one plate and two text-figures)

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I. Introduction

Of all the diseases of *pan* (*Piper betle*) the *Phytophthora* foot-rot and leaf-rot have been found to be the most serious. These diseases have been found widespread and have been observed in all the *pan*-growing tracts except the Satgaon-Athangiri tract where these diseases have been found completely absent. They are particularly severe during the months of heavy rainfall and are almost absent during the dry seasons and winter. Usually they appear with the first heavy monsoon showers in May and continue throughout the rainy season. The diseases are not at all noticed during the months from October to April.

Both the diseases appear to develop suddenly but it is doubtful if this is really so in all cases. Under moist conditions of high humidity and low temperature the development of the diseases may be rapid but they become inactive and the progress is slow when the season is dry and warm.

They attack plants in all stages of their growth. On the Manu, Kusiara and Surma banks the diseases appear in more or less serious proportions and the damage may be as high as 27·2 to 78 per cent. in many of the *borojes*. In certain *borojes* complete destruction of all the plants during a week has also been observed. In the Baraigram tract the diseases have caused very heavy losses and almost all the growers in this area have been compelled to give up the cultivation of *pan*. These diseases have also been found serious in the Satyajuri, Shaistaganj and Chunarughat tracts; the extent of damage has been found to range from 18·7 to 67·8 per cent. Everywhere the diseases have been found a serious menace to the economic growing of *pan*.

II. Symptoms

Foot-rot.—The very first symptom shown by a diseased plant is the loss of lustre. The leaves droop while still green or turn yellow and droop.

If the stem is examined at this stage blackish brown marks will be visible about an inch or so above the ground level or slightly below it. At this point the tissues of the stem are discoloured externally and internally. Later on the roots and stem disintegrate and give out a peculiar odour. In some cases the disease attacks the vines about a foot or two above the ground level and the parts above this area are killed.

Leaf-rot.—This has been found more serious than foot-rot and is observed under very wet conditions. The extent of the loss caused by this disease depends upon the prevailing climatic conditions. At times the disease may be in an epidemic form especially when there are long periods of rain and cloudy days, sunny days being few and far between. These conditions are favourable for the development of zoospores which are readily splashed from the infected soil surface against the low hanging leaves by the rain water. Leaves within a couple of feet from the ground surface are particularly liable to infection. The topmost leaves are seldom found diseased.

The first symptom of the disease is the presence of a circular blackish or brown spot which is more or less wet in appearance. Under continuous wet conditions the black spot readily increases in diameter and develops a wet rot of the major part of the leaf; the rot extends to the petiole and from the petiole to the stem. If wet conditions are not continuous and if the rain lasts only for a day or two with dry warm periods between the diseased areas show distinct concentric puckerings on account of an unequal growth of the tissues. Under dry conditions the progress of the disease is checked and the black spot usually surrounded by a brown ring remain dry and localised (Plate I). More than one spot are often found on a leaf; the spots may coalesce.

III. Etiology

A large number of isolations were made from diseased specimens collected from the different *pan*-growing tracts. A species of *Phytophthora* was always obtained in culture. The pathogenicity of the fungus was established in the usual manner by isolation, culture, inoculation, reisolation and comparison.

The morphological characteristics of the fungus was studied in culture and comparing it with a type-culture of *P. parasitica* Dastur obtained from the Imperial Mycologist, Imperial Agricultural Research Institute, New Delhi, it was identified to be a strain of *P. parasitica* Dastur.

IV. Temperature Relationship

The linear rate of growth of the fungus was studied on oat agar. Equal quantities of the medium were poured in petri-dishes of equal size and they were inoculated and incubated at 12°, 16°, 20°, 24°, 28°, 32°,

35° and 37° C. The experiment was run in triplicate. The diameter of the colonies were measured every day. The data are shown in Figs. 1 and 2.

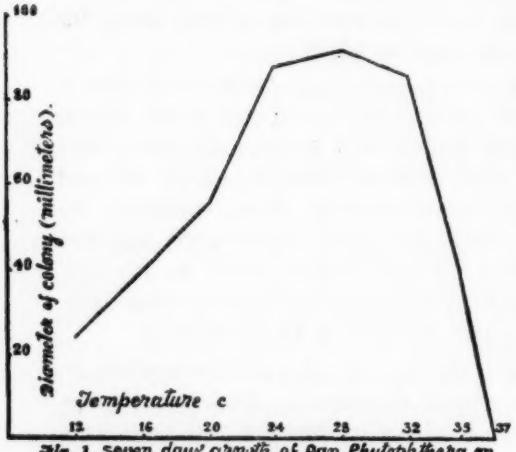
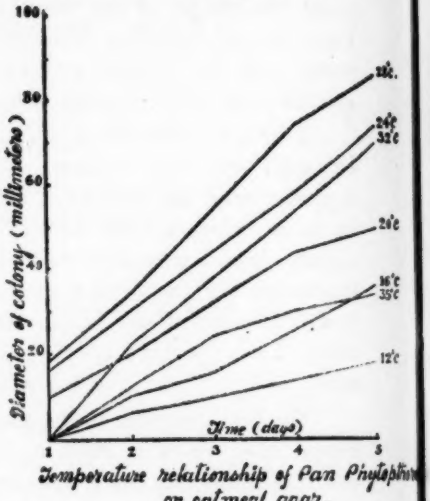


Fig. 1. Seven days' growth of Pan Phytophthora on oat agar at various temperatures.



Temperature relationship of Pan Phytophthora on oatmeal agar.

Fig. 2.

It will be observed from Figs. 1 and 2 that growth at 28° C. was always ahead of that at other temperatures and this is about the optimum for the growth of the fungus. At and above 37° C. there was no growth.

The thermal death point of the fungus was also found out and it was observed that five minutes' exposure at 48° C. kills the *pan Phytophthora.*

During the monsoon when the diseases are prevalent and do the greatest damage the prevalent minimum and maximum temperatures are within the range at which the fungus grows well. For instance during its active parasitism the daily temperature has been found to vary from minimum 21·6° to maximum 35° C., the average being 29° C. This agrees with the range for good growth of the fungus observed during its temperature relationship studies.

V. Modes of Perpetuation and Dissemination of the Disease

The following experiments were conducted to study the modes of perpetuation and dissemination of the disease:

(i) *Planting of healthy and unhealthy setts on disease-free soil.*—Two *borojes* were raised on a piece of land at Sylhet where *pan* had never been grown anytime in the past. Round-about these *borojes* within a radius of 20 miles there is no *pan boroj.* Besides the parasite has not been known or found to attack any other host in the locality. Each of these *borojes*

were divided into seven plots, each plot had eight rows and in each row six plants were planted.

In one of the *borojes* only healthy setts obtained from a *boroj* where the disease is entirely absent were planted. In the other setts obtained from a *boroj* where the disease was present were planted. In both the cases the setts were selected from the respective *borojes* at random. The results of the observations are recorded in Table I.

TABLE I

Observations on the planting of healthy and unhealthy setts on disease-free soils

Treatment	Plot	Number of plants planted	Number of deaths	
			1941–42	1942–43
Setts for planting selected at random from a *boroj* where the disease was found very serious	1	48	21	12
	2	48	17	19
	3	48	7	9
	4	48	19	14
	5	48	32	7
	6	48	29	9
	7	48	16	19
Setts selected at random from a *boroj* where the disease was entirely absent	1	48	nil	nil
	2	48	,,	,,
	3	48	,,	,,
	4	48	,,	,,
	5	48	,,	,,
	6	48	,,	,,
	7	48	,,	,,

Planting was done in April 1941 and the disease was first observed in July 1941 immediately after heavy showers during a period when the temperature was 30° C. and the relative humidity 98. The dead plants were microscopically examined and *P. parasitica* obtained from them in all cases of the isolations made.

(ii) *Planting of healthy setts on naturally infected soils.*—A *boroj* at Basudevsri where the disease was found very serious and where practically all the plants died of disease was selected. Following the method of Meredith (1940) *P. parasitica* was isolated from the soil. The *boroj* land was divided into eight plots, each plot having ten rows. Four of these plots received an additional dose of infection through fungal cultures which were incorporated with the soil before planting. Healthy setts were obtained from a *boroj* where the disease was absolutely absent and planted in May 1942, six setts in each of the rows. The results of the observations made are recorded in Table II.

TABLE II

Observations on the planting of healthy setts on diseased soil

Treatment	Plot	Number of plants planted	Number of deaths	
			1942	1943
Naturally infected soil	1	60	24	14
	2	60	18	32
	3	60	34	17
	4	60	29	22
Naturally infected soil receiving an additional dose of infection through fungal cultures	1	60	19	25
	2	60	44	12
	3	60	14	38
	4	60	26	21

(iii) *Planting of healthy setts on disease-free soils infected with infested soils.*—Four bags of soil were carried to Sylhet from a badly diseased *boroj* at Basudevsri. Cultures made from this soil revealed that it contains the parasite *P. parasitica.* A piece of land where experiment (i) was carried out and was free of infestation was selected. It was divided into two parts. The soil of one part was contaminated with the diseased soil brought from Basudevsri and the other portion was left untreated. Carefully selected healthy setts were planted. Each part was divided into four plots, each plot had ten rows and each row contained six plants. The planting was done in April 1942. The observations made are recorded in Table III.

TABLE III

Observations on the planting of healthy setts on disease-free soil contaminated with infested soil

Tre..tment	Plot	Number of plants planted	Number of deaths	
			1942	1943
Disease-free soil contaminated with infested soil	1	60	27	14
	2	60	20	17
	3	60	14	29
	4	60	12	17
Disease-free soil	1	60	nil	nil
	2	60	,,	,,
	3	60	,,	,,
	4	60	,,	,,

The dead plants were always microscopically examined and culture taken from them. In sections the characteristic phycomycetous mycelium could be seen and in culture *P. parasitica* could be obtained in all cases.

(iv) *Healthy setts planted on infested soil after sterilization.*—Infested soils were obtained from a very badly diseased *boroj* at Basudevsri. Many isolations were made from this soil and *P. parasitica* could easily be isolated from this soil. Earthen pots were filled with this soil and then sterilised for three hours at 20 lbs. pressure. Healthy *pan* setts were then planted in these soils. All the plants were healthy but healthy setts planted in soils of the same lot but not sterilized were diseased. The details are given in Table IV.

TABLE IV

Planting of healthy setts on sterilized and non-sterilized soils

Treatment	Number of pots	Number of plants	Number of deaths	
			1942	1943
Infested soil sterilized in autoclaves for three hours at 20 lbs. pressure	64	128	nil	nil
Infested soil not sterilized ..	64	128	76	37

(v) *Healthy setts grown on disease-free soil and afterwards contaminated with infested soil.*—Healthy setts were planted in a disease-free soil at Sylhet in 1941. During the first year no disease could be noticed and all the plants were very thrifty and healthy. During the second year in early May 1942 just before the monsoon rain infested soils were obtained from a badly infected *boroj* at Maulvibazar. Half of this soil was sterilized at 20 lbs.

TABLE V

Appearance of the disease due to the addition of infested soils

Treatment	Plot	Number of healthy plants	Number of deaths
Addition of infested soil at the base of healthy plants	1	42	27
	2	42	37
	3	42	29
	4	42	21
Infected soil sterilized and then placed at the base of healthy plants	1	42	nil
	2	42	,,
	3	42	,,
	4	42	,,
Plants and soil receiving no treatment ..	1	42	nil
	2	42	,,
	3	42	,,
	4	42	,,

pressure for three hours. The infested and the sterilized soils were then placed at the base of the healthy plants. Immediately after the first rains during a period of high humidity the disease appeared in the portion where the infested soil was added. The portion which received the sterilized soil was entirely free from any disease. The results are recorded in Table V.

From the above experiments it is concluded that the foot-rot and leaf-rot diseases of *pan* due to *Phytophthora parasitica* Dastur perpetuate and are carried away from one locality to another or from one *boroj* to the other in the same locality by the following methods:—

(*a*) The fungus can live in the soil. It is not possible to say how long the fungus can survive in the soil in the absence of its host plant, *pan*. In Basudevsri, Maulvibazar, Karimganj, Satyajuri, Shaistaganj and other places the *pan boroj* soil, in case of very heavy infestation by the disease causing germ, is allowed to remain fallow for three or four years but when the soil is replanted even after it has rested for three or four years the crop is again as much diseased as the previous crop, three or four years back. Either the parasite has remained dormant during this period or had led a saprophytic life in the soil.

(*b*) The fungus can live as a dormant mycelium inside planting setts and it is through diseased setts that the disease is primarily carried from one locality to another. In the same locality too it is one of the methods by which the disease is disseminated.

(*c*) In the same locality the disease may spread also through drainage water passing from one *boroj* to the other and also through contaminated soil. In this connection it may be stated that after each manuring with oilcake it is a common practice with the *baruis* to cover the manure with new earth brought from outside the *boroj*, either from river beds or from deposits of washings from the *boroj* soils during the rains. This new earth may become a focus of fresh infection and contaminate the *boroj* soil which was otherwise free of disease.

VI. Control and Prevention

(*a*) *Control*—

Experiments for controlling the diseases were carried out in the cultivators' *borojes* at Basudevsri, Shaistaganj, Rattibhallabpur and Purangaon and also at the experimental plot at Sylhet for the last three years. *Borojes* indicating very severe outbreak of the diseases were selected for treatment. The *borojes* were divided into randomised blocks and given the following treatments. Each treatment had six to eight replications.

The treatments were:—

A. Plants were left untreated. They served as controls.

B. The rotten leaves were picked and the diseased plants completely uprooted and destroyed. The plants were then sprayed and irrigated along the ridges with 2:2:50 Bordeaux mixture once a month. Every time before fresh spraying rotton leaves and diseased plants were removed and destroyed. One gallon of the mixture was applied per ridge 5 feet in length.

C. Plants were simply sprayed and irrigated with 2:2:50 Bordeaux mixture without picking up the rotten leaves and removing the dead plants. The rate of application was one gallon of the mixture for a ridge 5 feet in length.

D. All the rotten leaves were picked and diseased plants uprooted and destroyed. The plants were then sprayed and irrigated along the ridges with 2:2:50 Bordeaux mixture once in two months. One gallon of the mixture was applied per ridge 5 feet in length.

For economy of space the experimental results obtained at Basudevsri and Rattibhallabpur during the years 1941–42 and 1942–43 respectively are presented in Tables VI and VII. It may be mentioned in this connection that the results obtained at other places agreed with the results obtained at Basudevsri and Rattibhallabpur. In each case spraying was commenced in May and ended in September. At the time of the second treatment there was a distinct change in the general appearance of the plants sprayed and irrigated with Bordeaux mixture. In a great many cases no leaf-rot or foot-rot could be noticed even after the first treatment; wherever this was present it was found negligible in comparison with the untreated plots. The ridge which before treatment looked sparsely planted and had dull yellow coloured plants were now very full and thickly planted and full of green shining leaves. The plants which had been cut at the soil level developed new shoots from the eyes of the underground shoots. It is on this account that in certain cases an increase in the number of plants in the Bordeaux mixture treated plots have been noticed. The leaves were distinctly greener than normal healthy leaves of untreated plants.

From the data presented in Tables VI and VII the following conclusions can be drawn:—

(*a*) That the foot-rot and leaf-rot diseases can be controlled by spraying the *pan* vines and irrigating the plants along the ridges with 2:2:50 Bordeaux mixture.

TABLE VI

Results of experiments done for controlling the diseases at Basudevsri (1941–42)

Treatments	Replications	Total No. of plants on May 15, at the time of 1st spraying	Dates of subsequent spraying and the number of deaths noticed on those days				Total No. of deaths	Percentage of death	Average percentage of death for the treatment
			June 15	July 15	Aug. 15	Sept. 15			
A	1	227	57	62	27	7	153	67·4	56·86
	2	220	39	51	23	11	124	56·3	
	3	224	41	47	29	11	128	57·1	
	4	227	50	69	21	9	149	65·6	
	5	230	31	43	26	17	117	50·8	
	6	221	49	56	19	12	136	61·5	
	7	224	29	39	27	16	111	45·1	
	8	227	36	44	26	10	116	51·1	
B	1	232	4	1	..	..	5	2·1	1·81
	2	227	6	1	..	..	7	3·0	
	3	230	..	2	1	..	3	1·3	
	4	220	3	..	1	..	4	1·8	
	5	229	5	..	..	..	5	2·1	
	6	220	1	1	..	..	2	0·40	
	7	218	..	2	..	..	2	0·91	
	8	227	9	..	1	..	10	4·8	
C	1	221	10	4	4	..	18	8·1	9·48
	2	226	8	4	5	2	19	8·4	
	3	229	10	7	6	3	26	11·3	
	4	220	6	3	..	5	14	6·3	
	5	218	7	8	7	1	23	10·5	
	6	222	13	4	5	2	24	10·9	
	7	221	4	10	6	..	20	9·0	
	8	227	10	8	7	1	26	11·4	
D	1	231	..	22	..	7	29	12·5	11·67
	2	227	..	18	..	8	26	11·4	
	3	222	..	14	..	6	20	9·0	
	4	218	..	18	..	14	32	14·6	
	5	226	..	12	..	5	17	7·5	
	6	220	..	17	..	12	29	13·1	
	7	220	..	21	..	5	26	11·8	
	8	222	..	19	..	11	30	13·5	

(*b*) That the spraying should commence in May and be done once every month. The results obtained by spraying and irrigating the plants once every month were found more satisfactory than by spraying and irrigating the plants with Bordeaux mixture once every two months. This is probably due to the fact that due to heavy rainfall in the district during this period the chances of the spraying fluid getting washed away is more and hence more frequent spraying is a necessity.

TABLE VII

Results of experiments done for controlling the diseases at Rattibhallabpur (1942–43)

Treatments	Replications	Total No. of plants on May 12, at the time of 1st spraying	Dates of subsequent spraying and the number of deaths noticed on those days				Total No. of deaths	Percentage of death	Average percentage of death for the treatment
			June 12	July 12	Aug. 12	Sept. 12			
A	1	224	47	62	27	19	155	69·1	64·5
	2	221	39	51	21	12	123	55·6	
	3	234	42	57	29	11	139	59·4	
	4	220	51	69	33	13	166	75·4	
	5	219	46	59	29	16	150	68·4	
	6	230	41	52	26	17	136	59·1	
B	1	229	4	1	..	..	5	2·1	1·28
	2	230	..	2	1	..	3	1·3	
	3	218	2	..	..	..	2	0·9	
	4	227	3	..	..	..	3	1·3	
	5	229	..	2	2	..	4	1·7	
	6	231	..	1	..	..	1	0·4	
C	1	218	7	9	4	2	22	10·0	9·08
	2	229	6	7	5	1	19	8·2	
	3	217	4	6	2	..	12	5·5	
	4	230	11	8	4	4	27	11·7	
	5	230	5	10	3	3	21	9·1	
	6	228	6	8	5	4	23	10·0	
D	1	229	..	12	..	5	17	7·4	8·46
	2	227	..	11	..	6	17	7·4	
	3	218	..	16	..	2	18	8·2	
	4	231	..	15	..	7	22	9·9	
	5	226	..	19	..	1	20	8·8	
	6	230	..	17	..	4	21	9·1	

(*c*) That removal and destruction of diseased leaves and plants before spraying is essential. It will be observed from the data that spraying was more effective when diseased leaves and plants were removed and destroyed than when they were not removed and destroyed. It is very probable that these diseased materials serve as sources for fresh infection.

(*b*) *Prevention*—

From a consideration of the studies made under modes of perpetuation and dissemination of the disease it will become quite clear that in order to prevent the disease the following methods will have to be adopted:

(i) It has been found that the parasite can live in the soil and when a crop is planted on a diseased land it is completely wiped out by the disease

before it is a year or two old. Infected soils should therefore be sterilized before planting. This can be done either by heat or by chemicals.

(ii) After sterilizing the soil only healthy setts should be planted for it has been observed that the disease can be disseminated through diseased setts. Healthy setts can be obtained from *borojes* where the disease is entirely absent.

(iii) Subsequent infection through soil or drainage water from adjacent diseased *borojes* should be guarded against. The new earth that is applied every year after manuring should preferably be treated with Bordeaux mixture before it is applied, or the application of new earth on the ridges should immediately be followed by irrigation with Bordeaux mixture.

The following experiments were carried out to find the effect of soil sterilization on the prevention of *pan* diseases. A *boroj* where all the plants have died of disease was selected at Raikail. A large number of isolations were made from this soil following the method of Meredith (1940) and in each case *P. parasitica* was isolated. An additional dose of infective material was incorporated into the soil by the addition of fungal cultures grown on oat agars. The land was divided into eight equal blocks and each block into four equal plots. They were then given the following treatments and each treatment had eight replications.

A. Soils left without any treatment. They served as control.

B. Soils burnt during very hot sunny days by means of rice straw, thatching grass, etc. The land was first dug 8–10 inches deep with a spade. Straw and thatching grass were then spread on it and lighted. The burning continued for 20 minutes. Immediately after the cessation of the fire the temperature of the soil was taken upto the depth of 10 inches. It was found to be 60–70° C. During the process of burning the temperature might have been surely more than this. This temperature is much more than the thermal death point of the fungus which has been found to be 48° C. The burning operations were carried out four times, turning the soil every time after each burning.

C. Soils treated with 2:2:50 Bordeaux mixture. The soil was made loose by hoeing and then Bordeaux mixture sprayed from a sprayer till the soil was saturated. 25 gallons of the mixture was used for every 100 sq. feet of land.

D. Soils treated with kerol (1 part in 600 parts of water). The soil was hoed and then the solution sprayed from a sprayer till the soil was saturated. 20 gallons of the mixture was used per 100 sq. feet of land.

A fortnight after these treatments equal number of healthy setts (60 setts) were planted in each of the plots. The setts were very carefully selected from an entirely disease-free *boroj*. The planting was done in May 1941. Observations were made every month and the number of deaths noted. The number of deaths noted till May 1943 are recorded in Table VIII.

TABLE VIII

Number of deaths observed in blocks treated with heat and chemicals

Treatments	Number of plants in each block	Number of deaths in each block								Total No. of plants	Total No. of deaths	Percentage of death
		1	2	3	4	5	6	7	8			
Heat ..	60	2	..	1	..	..	2	..	..	480	5	1·02
Bordeaux mixture	60	..	2	..	2	..	..	3	1	480	8	1·66
Kerol ..	60	1	2	..	2	1	4	..	..	480	10	2·08
Control ..	60	29	31	37	38	43	41	39	40	480	298	62·83

It will be observed from the data presented in Table VIII that infected soils can be effectively sterilized by heat, Bordeaux mixture and kerol and the percentage of death very much reduced.

Soil sterilization experiments were also conducted at Maulvibazar. One very badly diseased *boroj* was selected. It was divided into sixteen equal plots. Soils of eight of these plots were burnt very thoroughly by rice straw and thatching grass by turning the soil over again and again; the remaining eight plots were left without treatment to serve as control. Equal number of healthy *pan* setts (72 setts) were planted in each of these plots in June 1941. Observations were made every month and the number of deaths of plants noted. At the close of the second year, *i.e.*, in June 1943 it was found that no death had occurred in the heat-treated plots whereas in the eight control plots 517 plants had died in all out of a total of 576 plants and the surviving ones were in a very sickly state of health.

In sterilizing the soil it is necessary that the operation be done very thoroughly so that no part of the soil escapes the treatment. Success will depend on the thoroughness of the treatment.

VII. Summary

Phytophthora foot-rot and leaf-rot are the most serious diseases of *pan* in the district of Sylhet. They are found all over the *pan*-growing tracts except the Satgaon-Athangiri tract.

The diseases attack plants in all stages of their growth and the damage done may be as high as 30 to 50 per cent. in many of the *borojes*. In certain areas they have caused such heavy losses that cultivators have been compelled to give up the cultivation of *pan*.

The symptoms of the foot-rot and leaf-rot diseases are described. The diseases appear usually in May and continue till September. The greatest damage is done during long periods of rainy and cloudy days.

The causal organism is *Phytophthora parasitica* Destur.

Temperature relationship studies have been made and it has been found that the optimum temperature for the growth of the fungus is 28° C. It has also been observed that five minutes' exposure at 48° C. kills the fungus.

Modes of perpetuation and dissemination of the disease have been studied. It has been found that the fungus can live in the soil. As dormant mycelium inside the planting setts it may be carried from place to place, and through drainage water and contaminated soil it may spread in the same locality.

Methods of prevention and control have been studied. It has been found that the diseases can be controlled by spraying the plants and irrigating them along the ridges by 2:2:50 Bordeaux mixture once a month during the rains, commencing from May and ending in September.

For prevention it has been found necessary to sterilize the soil before planting either by heat or by chemicals. Heat, Bordeaux mixture and kerol have been tried. It has been found that the disease can be prevented by burning the soil thoroughly. In all cases it is necessary that after sterilization of the soil only healthy setts should be planted and fresh infection through diseased soil or drainage water guarded against.

Acknowledgements

My thanks are due to Dr. G. W. Padwick, Imperial Mycologist, Imperial Agricultural Research Institute, New Delhi, for going through the manuscript and for making many valuable suggestions. I am also grateful to Mr. S. Majid, formerly Deputy Director of Agriculture, Sylhet, for facilities offered and keen interest taken in this work.

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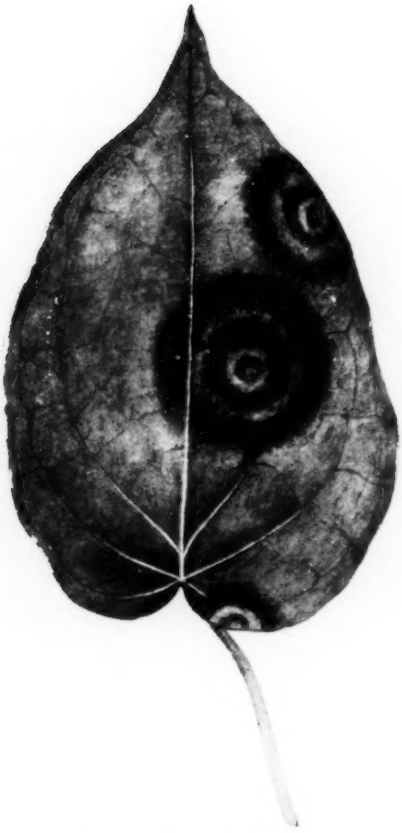

Symptoms of the Disease

DISEASES OF PAN (*PIPER BETLE*) IN SYLHET, ASSAM

III. Effect of Manuring on the Incidence of *Phytophthora* Foot-rot and Leaf-rot Diseases

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Received December 10, 1943

(Communicated by Dr. H. Chaudhuri, D.SC.)

I. Introduction

THE manure that is normally and primarily used for *pan* (*Piper betle*) is mustard oil-cake. It is ground fine, spread along the ridges and then covered with a very thin dressing of soil. No definite weight is taken of the quantity applied; but information gathered shows that during the first year of planting oil-cake is applied at the rate of 12 maunds per acre and during the subsequent years it is applied at the rate of 36 maunds per acre.

Manuring is done only during the rains, once a month commencing from May and ending in September or October. Equal quantities of manures are applied at each application. In all usually five or six applications are made during a year.

Oil cake is an organic manure and it was presumed that it might serve as a nutrient medium for the growth of *Phytophthora* and might influence the incidence of *Phytophthora* foot-rot and leaf-rot diseases of *pan* and that the virulence of the diseases might be checked or minimised by replacing oil-cakes by fertilizers. Had this been possible it might have been possible to control the disease to a certain extent by this indirect method.

In order to test the truth of this assumption and the possibility of checking completely or partly the virulence of the disease by manuring the plants with fertilizers an experiment was carried out. The results of this experiment are recorded in this paper.

II. Materials and Methods

The experiment was laid out in a *boroj* land at Rattibhallabpur where all the plants have died of the diseases and where by soil isolation studies according to the method of Meredith (1940) it was found that the soil was uniformly infested with the parasite, *Phytophthora parasitica*, Dastur.

The entire plot was divided into eight blocks and each block into five plots of equal size. Each plot was 30 × 6 feet. There were twenty rows in

each plot and ten setts were planted on each row. Planting was done on 7th October 1940 and only healthy setts were planted.

The first manuring was done during the later part of April 1941 and the subsequent manurings during the months May, June, July, August and September 1941.

During the second year manuring was commenced in May 1942 and ended in October 1942. Six applications were made, one application in each of the months May, June, July, August, September and October.

In every case the manuring was done the same day and at the same time. The method of application was also the same. The oil-cake was ground very fine, then spread along the ridges and afterwards covered with a thin layer of fine earth. The fertilizers, on the other hand, were mixed with an equal quantity of fine earth, thoroughly mixed together and then applied along the ridges just like oil-cake and then covered with a thin layer of soil.

The treatments were as follows:—

A. *Oil-cake.*—Applied at the rate of 984 lbs. per acre during the first year, 1941 and at the rate of 2952 lbs. during the second year, 1942. Samples of the oil-cake applied were analysed and found to contain on an average 5·1 per cent. nitrogen, 3·13 per cent. phosphoric acid and 1·35 per cent. of potash.

B. *Sodium nitrate.*—Applied at the rate of 321 lbs. per acre during the first year, 1941 and at the rate of 963 lbs. during the second year, 1942. The samples were found to contain on an average 15·6 per cent. of nitrogen.

C. *Ammonium sulphate.*—Applied at the rate of 242 lbs. per acre during the first year, 1941 and at the rate of 726 lbs. per acre during the second year, 1942. The samples were found to contain on an average 21·0 per cent. nitrogen.

D. *Mixture of Sodium nitrate, Superphosphate and Potassium sulphate.*—A mixture containing 321 lbs. of sodium nitrate, 200 lbs. of superphosphate and 25 lbs. of potassium sulphate was applied per acre during the first year, 1941 and a mixture containing 963 lbs. of sodium nitrate, 600 lbs. of superposphate and 75 lbs. of potassium sulphate during the second year, 1942. Superphosphate was found to contain 16 per cent. of phosphoric acid and potassium sulphate 48 per cent. of potash.

E. *Mixture of Ammonium sulphate, Superphosphate and Potassium sulphate.*—A mixture containing 242 lbs. of ammonium sulphate, 200 lbs. of superphosphate and 25 lbs. of potassium sulphate was applied per acre

TABLE I

Effects of manuring on the incidence of Phytophthora *foot-rot and leaf-rot diseases of pan* (1941)

Treatment	Pounds of fertilizing ingredients applied per acre			Replication	Total number of setts planted	Number of deaths during the year	Percentage of death	Average percentage of death for the treatment	Yield of leaves	Average yield of leaves for the treatment
	N_2	P_2O_5	K_2O							
A	50·184	30·799	13·284	1	200	72	36	29·25	6141	6785·37
				2	,,	58	29		6812	
				3	,,	62	31		6623	
				4	,,	56	28		6879	
				5	,,	38	19		7765	
				6	,,	48	24		7293	
				7	,,	72	36		6174	
				8	,,	62	31		6596	
B	50·76	..	..	1	200	52	26	30·00	7102	6727·87
				2	,,	76	38		5824	
				3	,,	74	37		6042	
				4	,,	58	29		6901	
				5	,,	48	24		7283	
				6	,,	54	27		7005	
				7	,,	80	40		5761	
				8	,,	38	19		7905	
C	50·82	..	..	1	200	62	31	29·75	6625	6727·25
				2	,,	58	29		6802	
				3	,,	62	31		6619	
				4	,,	58	29		6816	
				5	,,	74	37		6053	
				6	,,	54	27		7012	
				7	,,	44	22		7299	
				8	,,	64	32		6592	
D	50·76	32·0	12·0	1	200	38	19	30·50	7761	6728·12
				2	,,	80	40		5969	
				3	,,	74	37		6140	
				4	,,	62	31		6621	
				5	,,	58	29		6798	
				6	,,	62	31		6627	
				7	,,	54	27		7069	
				8	,,	60	30		6840	
E	50·82	32·0	12·0	1	200	60	30	29·62	6817	6727·12
				2	,,	62	31		6613	
				3	,,	68	34		6337	
				4	,,	54	27		7002	
				5	,,	78	39		5822	
				6	,,	52	26		7111	
				7	,,	58	29		6623	
				8	,,	42	21		7492	

TABLE II

Effect of manuring on the incidence of Phytophthora *foot-rot and leaf-rot diseases of pan* (1942)

Treatment	Pounds of fertilizing ingredients applied per acre			Replication	Total number of setts planted	Number of deaths during the year	Percentage of deaths	Average percentage of death for the treatment	Yield of leaves	Average yield of leaves for the treatment
	N_2	P_2O_5	K_2O							
A	156·55	92·40	39·85	1	128	31	24·1	27·17	5937	5324·12
				2	142	39	27·4		5130	
				3	138	34	24·6		5942	
				4	144	37	25·7		5753	
				5	162	50	30·8		4691	
				6	152	48	31·5		4442	
				7	128	34	26·5		5440	
				8	138	37	26·8		5259	
B	152·28	..	..	1	148	39	26·3	27·10	5239	5121·13
				2	124	27	21·7		6018	
				3	126	27	21·4		6027	
				4	142	41	28·8		5002	
				5	152	46	30·2		4230	
				6	146	39	26·7		5240	
				7	120	29	24·1		5712	
				8	162	61	37·6		3201	
C	152·46	..	..	1	138	49	35·5	27·10	3617	5126·75
				2	142	51	35·9		3605	
				3	138	32	23·1		5872	
				4	142	37	26·0		5247	
				5	126	29	23·0		5971	
				6	146	27	18·4		6524	
				7	156	49	31·4		4307	
				8	136	32	23·5		5871	
D	152·28	96·0	36·0	1	162	47	29·0	27·62	5012	5124·0
				2	120	41	34·1		3831	
				3	126	32	25·3		5640	
				4	138	36	26·1		5248	
				5	142	31	21·8		6017	
				6	138	37	26·8		5253	
				7	146	43	29·4		4989	
				8	140	39	28·5		5002	
E	152·46	96·0	36·0	1	140	28	20·0	27·46	6318	5122·12
				2	138	27	19·5		6593	
				3	132	31	23·4		5892	
				4	146	42	28·7		5102	
				5	122	39	31·9		4320	
				6	148	49	33·1		3929	
				7	142	44	30·9		4652	
				8	158	51	32·2		4171	

during the first year 1941 and a mixture containing 726 lbs. of ammonium sulphate, 600 lbs. of superphosphate and 75 lbs. of potassium sulphate during the second year, 1942. The percentages of phosphoric acid and potash were the same in superphosphate and potassium sulphate respectively as in D.

III. Experimental Results

After manuring the number of deaths occurring in each of the plots was noted and are recorded in Tables I and II for the years 1941 and 1942. The yield of leaves was also ascertained and is recorded in Tables I and II. The dead plants were collected and examined; it was found that in all cases the deaths occurred due to the attack of the parasite *Phytophthora parasitica* Dastur.

It will be observed from the data presented in Tables I and II that the percentage of death is practically the same for all the treatments, there being no significant difference. As regards the yield of leaves it will be found that it is slightly more in the oil-cake treated plots during both the years than in the plots treated with the artificials.

As to the general growth of the plants due to manuring it was found that the effects of the artificials were earlier visible on the plants than oil-cake but in the long run no difference could be noticed between the plants given the different treatments; the height of the plants the size and colour of the leaves and the general vigour of the plants were indistinguishable. But during the cold weather and early part of the dry season (November to April) it was found that the oil-cake treated plants were in a better state of health and thriftiness than those receiving the artificials. This is probably due to the fact that oil-cake increases the organic matter and humus content of the soil and thus helps the conservation of moisture. Further the food materials contained in oil-cake become gradually and slowly available to the plants; thus its effects are more lasting than the effects of the artificials. Another distinct advantage of oil-cake over artificials was also noticed; it was found that in a region of heavy rainfall like Sylhet the chances of the manure getting washed away by the heavy showers is more with respect to the artificials than with oil-cake.

IV. Conclusions and Summary

As a result of the experiments conducted with different manures it was found that the percentage of death of *pan* plants due to the attack of *P. parasitica* is not influenced by the nature of the fertilizing material. The percentage of death has been found to be practically the same in the plots receiving oil-cake, sodium nitrate, ammonium sulphate singly or sodium nitrate

or ammonium sulphate in combinations with superphosphate and potassium sulphate. The yield of leaves, however, has been found slightly more in the oil-cake treated plots.

My thanks are due to Dr. G. W. Padwick, Imperial Mycologist, Imperial Agricultural Research Institute, New Delhi, for going through the manuscript.

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STUDY IN DISEASES OF FISH: INFESTATION OF FISH WITH LEECHES AND FISH LICE

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With the advent of rural pisciculture during recent years, there has been continually increasing demand for fish fry for stocking tanks and ponds. Difficulties of various kinds have, however, to be met and overcome if the fish are to be kept healthy and growing rapidly in these confined waters. One of these difficulties is to protect them from the various diseases which the fish in captivity are likely to fall a victim. Wild fish are rarely affected by diseases due mainly to conditions under which they live in nature. Even if the wild fish are attacked by any parasite, little or no injury is caused, but when the fish are confined in tanks and ponds, there is every opportunity of the rapid increase and spread of parasite organisms, resulting in the outbreak of epidemics with consequent heavy losses.

During recent experiments on propagation of carp in tanks at Lyallpur it was observed that in one of the tanks the carp, both young and adult, had been attacked with leeches, and in another tank, they were infested with fish lice. Study of these parasites, together with mode of their attack and of their control, is given in this article for the information of those who are engaged on rearing of fish in their tanks and ponds.

Leeches[1]

The attack of leeches on fish appear to be a rare phenomenon. Thompson (1927) described an epidemic of leeches on fishes in Rock River in the State of Illinois. But in India, so far as information is available an attack of leeches on fish has not previously been recorded except a casual reference by Southwell (1915, p. 324), that Kemp, during inspection of some diseased fish in a tank near Cossim Bazar, associated the disease " with scanty food and the presence of large number of leeches and parasitic copepods ".

It was, therefore, interesting to note that on the 14th June 1939, some young and adult carp, namely, *Cirrhina mrigala* (Hamilton) and *Labeo rohita* (Hamilton), taken out from a tank at Lyallpur, were found to be infested

[1] The author is indebted to Dr. M. L. Bhatia of Lucknow University, for identification of these leeches. They belong to the genus *Hemidepsis*, s.o. Rhyncholedellæ, fam. Glossiphonidæ.

with leeches, which had attached themselves mainly to softer parts of the fish, such as lips, nostrils, gills, axils of the fins and the anus. Both the species of the fish were equally affected (Figs. 1 and 2, L.). The size of the leech ranged from 3 to 25 mm. each (Fig. 3).

The fish infested with leeches were put in an aquarium and fresh water allowed to run. Next day, most of the leeches had left the fish and had crept under the gravel stones lying in the aquarium. The leeches were observed to attach themselves to the tips of the *Hydrilla* branches, planted in the aquarium and stretch them. Due to their speckled green colour and the way in which they attached themselves to the green plants, they appeared like leaves of *Hydrilla*. On first sight it was difficult to differentiate between the two, and when a fish passed near the plant many leeches stretched themselves and forced a hold on it by means of their anterior suckers. The number of leeches on the infested fish ranged from 5 to 230 on a fish.

It was, further, observed that the leeches in the aquarium attacked all kinds of fish, namely *Ophiocephalus punctatus* (Bloch), *Labeo microphthalmus* (Day), *Barbus* (*Puntius*) *sophore* (Hamilton), *Colisa lalia* (Hamilton), and *Carassius auratus*, L. Even frogs when introduced into the aquarium were not spared.

Mortality.—No mortality was noticed in the tank among the fish infested with leeches. But in the aquarium all the fish attacked by the leeches died within a week due to loss of blood sucked and of fungus growth on wounds caused, by the leeches. The fish were observed to be off the feed on the second day of the attack, and before their death they turned upside down. Their bodies were curved and coagulated blood was noticed on the fins where leeches had caused wounds. There was an abundant secretion of mucus where leeches had attached themselves. Internally the alimentary canal was totally empty.

Control.—Of the various solutions generally recommended for the control of external parasites, solution of glacial acetic acid proved successful in the treatment of fish in the aquarium. The usually recommended strength of glacial acetic acid of 1 : 500, was undoubtedly very effective in killing the leeches, but it killed the fish as well. A dilute solution of 1 : 1000 was found to kill the leeches in one minute without any harm to the fish itself. Disinfectation of the aquarium was done by a solution of one gram of potassium permanganate to 10,000 parts of water, which killed all the leeches in twenty-four hours. For the tanks the only remedial measure that can be recommended as an effective is cleaning, drying and refilling it with fresh water.

Fish Lice (Argulus)

So far as information is available, Southwell (1915) recorded *Argulus foliaceus* for the first time from India, though specimens of the species had previously been collected by Kemp and Annandale, and these have been referred to by Southwell in his paper. Kemp and Southwell obtained their material from Bengal and Bihar respectively from the skin of *Labeo rohita*. It was significant that the parasite was found on *Labeo rohita* only, though other carps, such as *Catla catla* and *Cirrhina mrigala* were found living in the same or adjoining tanks. In 1933, Hora recorded this parasite from the skin of *Ophiocephalus* living in a breeding tank in the Dharangadhara State.[2] Recently Hora (1943) described a heavy infestation of the parasite on *Labeo rohita, Catla catla* and *Cirrhina mrigala* in the Churchuria Fishery, Dhapa.

In October 1941, in one of the tanks at Lyallpur, one or two *Cirrhina mrigala* (Hamilton) were daily picked out dead. The fish were always in advanced stage of decomposition and no external parasite could be found on their body, although in many places the scales had fallen off and patches of naked skin were visible. One day a dying fish was caught from the tank and was examined. It was found to be heavily infested with fish lice (*Argulus*). The point of attack was mostly on the skin in the basis of the scales, which consequently had fallen off in patches exposing the body (Fig. 4, *s*.). As soon as the fish died, the parasite left it and swam about freely in search of new host. It was observed that *Labeo rohita* (Hamilton), living in the same tank, proved to be immune to the attack of fish lice as during this period not a single *Labeo rohita* died and all were found to be in healthy condition.

The present record of the parasite from the skin of *Cirrhina mrigala*, together with the observations made by Hora and previous authors, tends to show that the parasite is probably fairly widely distributed in India, though it has not received much attention from the pisciculturists, and is capable of infesting several species of fresh-water fishes.

Mortality.—Usually the parasite does not do much harm, but in tanks and ponds and hatcheries where the fish are crowded together it becomes a regular menace. In Bengal, Southwell (1915) pointed out that "The mortality amongst tank fish in particular, due to the presence of this parasite, is, in all probability, fairly extensive." In the present case, which is probably

[2] The author is indebted to Dr. S. L. Hora, Director of Fisheries, Bengal, for a copy of his unpublished article on "*Argulus foliaceus* (Linn.), a carp louse, infesting *Ophiocephalus* Bloch".

first of its kind recorded from the Punjab, the parasite had killed 19 out of 130 fish within three weeks, before any control measure could be adopted to check it.

Control.—In the case recorded by Southwell (1915), there were 18 mature specimens of *Labeo rohita* to be dealt with and each individual could be caught and scraped as clean as possible. An upright bamboo was then erected in the centre of the pond and " the fish proceeded forthwith to rub themselves against this bamboo. There were no deaths." In America and on the Continent the infested fish have been known to rub themselves against hard objects to dislodge the parasites and biological control of the pest is also attempted by introducing small fish such as roach, dace and bream (Southwell, 1915, page 325). For the fish in aquarium, Roughley (1933) recommends the picking off parasites with fine pointed forceps, or " they will drop off on their own accord if they are touched with kerosene or a mixture of equal parts of kerosene and turpentine on a camel hair brush." Tung-pai Chen (1933) recommends " previous sterilization of the ponds and the introduction of small fishes into the ponds." He also advises treatment of infected fishes with a solution of one part of ammonium chloride to one thousand parts of water. Dahlgren (1933) recommends that before stocking a hatchery pond the fish should be washed in a solution of potassium permanganate ($\frac{1}{2}$ grain to a gallon of water) for a maximum period of twenty minutes, and that the ponds may be disinfected with a stronger solution of potassium permanganate ($\frac{3}{4}$ grain to a gallon of water) twice or thrice if necessary after every ten days. He also recommends introducing harmless minnows such as dace in the pond after the treatment. Recently Hora (1943) observed that introduction of considerable amount of brackish water checked the epidemic caused by *Argulus* and that the fish showed no trace of parasites afterwards. In the case recorded from Lyallpur it was possible to cure the fish infested with *Argulus*, in early stages of its attack, by a bath of 1 : 1000 glacial acetic acid for 5 minutes followed by 1% salt bath for one hour. In advanced stages the cure was impracticable. Drying and cleaning the tank and filling it with fresh water succeeded in killing the parasites altogether. The introduction of larvivorous fish, such as *Colisa lalia*, *Ambassis baculis* and *Barbus* (*Puntius sophore* also helped in checking the reappearance of the parasite.

FIG. 1 ×½

Labeo rohita infested with leeches at L

FIG. 3. ×3

Hemiclepsis sp. Leech infesting fishes (dorsal and ventral views)

FIG. 2 ×½

Cirrhina mrigala infested with leeches at L

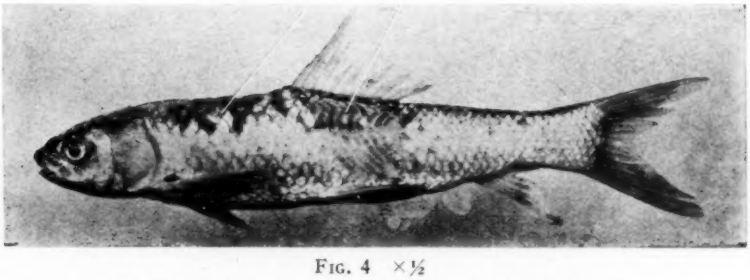

FIG. 4 ×½

Cirrhina mrigala infested with fish-lice (*Argulus*) at S.

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LIFE-HISTORY AND BIONOMICS OF POTATO TUBER MOTH (*PHTHORIMAEA OPERCULELLA* ZELL., *LEPIDOPTERA: GELECHIIDAE*)

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Introductory

Potato is an important money crop amongst vegetables in this Province as in other parts of the country. It is grown extensively in the Deccan and part of Karnatak. Storing the produce for seed purposes as well as for human consumption is a serious problem both with the cultivators and the stockists since the tuber moth—a notorious pest—causes a considerable damage to potatoes in storage. It is a pest of world-wide distribution and causes a lot of damage to various crops such as tobacco, tomato and potato tubers in other parts of the world. Its activity in India, however, is restricted to potatoes only. The damage in the store is well known but its presence in the field is not considered of great significance.

Since fumigation needs an air-tight chamber or a bin with special arrangements as well as petrol which is a rationed article these days, it is not a practical control measure with the cultivators. The present investigations, therefore, were undertaken during 1943, with a view to studying the life-history and bionomics of the pest under local conditions and also to determine some other control measures which may be more practicable and economical.

Historical

The earliest record from India is by Lefroy (1907) who gives its distribution in the country, and describes the duration of the various stages as studied at Pusa and records a maximum of 86 eggs as laid by a single female. Lefroy and Evans (1910) came to the conclusion that the pest was introduced with the potatoes imported from Italy about the year 1900. Picard (1912) observed parthenogenesis in a few cases and remarked that oviposition was

probably the result of rubbing of the abdominal end against any rough surface. Woodhouse (1913) is of the opinion that the pest does not cause any damage to the potato crop in the field. Kasergode and Nagpurkar (1920) studied its life-history at Belgaum during summer but their observations do not agree with those of ours with respect to the duration of the larval stages and the fecundity although the climatic conditions at Belgaum and Poona are almost identical. Husain (1926) recorded 13 generations during a period of 11 months at Pusa. Atherton (1936) compared the life-histories of *P. operculella Z.* and *P. heliopa.* Ghulamullah (1939) obtained on an average, 100 eggs per female under laboratory conditions at New Delhi. Rahman and Sohi (1940) described the life-history, nature and extent of damage and the preventive measures in the Punjab. Helson (1942) determined the efficacy of acetylene as a larvicide and also studied the relative value of various dusts, namely, derris, magnesite, dolomite and lime on oviposition and subsequently on the percentage of infestation.

The present investigations were taken up with a view to finding out some effective method of control through the application of some volatile substances which might ultimately produce detrimental effect on the multiplication of the pest under ordinary conditions of storage.

Technique

For mass breeding and study of relative infestation under different conditions as well as for estimating the effect of some of the volatile substances on the development of the larvæ, glass cells of 6″ × 4″ dimension were utilised. For the study of oviposition under different conditions, the newly emerged adults were kept in pairs in specimen tubes 3″ × 1″ with muslin at the mouth. The eggs were laid indiscriminately but mostly on the cloth. Liquids in the form of water or sugar solution were provided as a few drops when necessary because soaked cottton did not solve the problem. Hatching of eggs was observed in small petri dishes whereas for the larval stage, newly hatched larvæ were liberated in sound potatoes and reared in glass jars measuring 4″ × 3″ with the mouth covered with muslin cloth. The pupæ, however, were separated in specimen tubes. To study the relative effect of volatile substances on oviposition and hatching of eggs, the pupæ and the eggs were kept in petri dishes in the presence of those substances and the adults removed for oviposition as soon as emerged.

Description of Stages

Egg.—Slightly oval, basal portion rather flattened, apparently smooth, white when fresh, changes to brown after about 24 hours and ultimately to blackish before hatching; average measurements 0·40 × 0·28 mm.

Larva (*newly hatched*).—White; head black; prothorax the broadest, with three pairs of setæ on the anterior half and one or two towards the posterior border; other thoracic segments each with two pairs of setæ; abdomen consists of ten visible segments.—Each abdominal segment except the 9th provided with 2 pairs of setæ on the anterior border and one on the posterior. Each thoracic as well as abdominal segment except the 10th has a lateral tubercle on each side from which arise three setæ in the case of the 9th abdominal segment and the prothorax while two in other cases. Four pairs of prolegs, each provided with three prominent setæ at the base. A pair of setæ, one on each side of the mid-ventral line, is situated on all the thoracic and abdominal segments except the 10th. Laterally on the prothorax, 1st, 2nd and the 7th abdominal segments are two setæ arising on tubercles. The legs are small and end in single claws.

The larva measures 1·43 mm. × 0·21 mm. the head being 0·16 × 0·19 mm.

Full-grown larva.—The full-grown larva measures on an average, 10·21 mm. × 1·09 mm. It is the widest at the metathorax and tapers both ways; body colour is pink dorsally, changes subsequently to greenish in most cases, whitish at the inter-segmental sutures, greyish laterally and on the underside, meso-thorax pink or greenish. Head snuff coloured, black patch at the posterior margin of the prothorax, divided into two halves. Legs brown, spines as described previously. The larva moults thrice during its life. The head measurements of the respective instars are 0·19, 0·34, 0·59 and 0·93 mm.

Pupa.—Obtect, barrel-shaped, narrower posteriorly, minutely hairy, measures 6·47 mm. × 1·89 mm. at the broadest region; head deflexed ventrally; newly formed pupa is greenish on the head and thoracic region, the rest of the body whitish-green dorsally, and uniform green ventrally. The colour changes subsequently to brown or straw colour with a few markings. Abdominal end provided with a dorsal, recurved horn with two prominent bristles laterally and a few short ones on the horn itself.

Adult.—Greyish, wing span 13·5 to 16 mm., length 5·6 mm., scales grey, blackish-grey, or greyish-brown. Eyes prominent, black; antennæ with black and grey bands, in repose held back along the body; first pair of legs poorly developed, tibial spurs wanting, the third pair the longest, with two pairs of peg-like tibial spurs—a pair at the tip and the other in the middle; tarsus 5 jointed ending in paired claws and pulvillus in between; abdomen tapering posteriorly and ending in the male, in a plume of hairs the outer being longer than the inner and arranged to form a fan-like

structure under which lie the genitalia. In female, the abdomen terminates in a tubular orifice (Figs. 1 and 2).

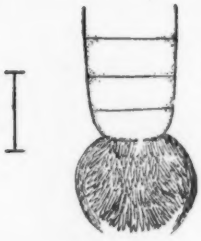

FIG. 1
Abdominal Tip of Male

FIG. 2
Abdominal Tip of Female

Life-History

Eggs are laid in groups at the eye buds and the duration of the egg-stage during summer lasts for 3 days (temperatures 91·4 – 93·5° F. max. and 81·4–84·7° F. minimum) and 4 days during the monsoons (temperatures 81·0–84·9° F. max. and 76·6–78·5° F. minimum). After hatching, the larva tunnels inside the tuber and feeds on the pulp, and the presence of excreta on the potatoes indicates their infested condition. The larval stage varies from 9 to 15 days, with an average of 11·1 days. In most cases the full-grown caterpillar finds its way out of the tuber usually two days before pupation. After selecting a suitable place especially between the potatoes, within rubbish or in the cracks, the larva pupates in a cocoon. The pupal stage varies from 4 to 7 days and on an average, occupies 5·25 days. The whole life-cycle varies from 16 to 26 days with an average of 18·3 days.

Oviposition and span of life (Fig. 3) under—

(1) *Normal conditions.*—Usually the moth starts laying eggs on the next day after emergence, but in some exceptional cases the pre-oviposition period may extend to 48 hours after the emergence. In the absence of any food, each female is capable of laying on an average, 76 eggs in four days. The maximum number of eggs under these conditions was 110 and the average life extended to 5·79 days in female and 4·84 days in the male. The highest number of eggs is laid invariably on the second day.

(2) *Supply of water.*—(*a*) When water was supplied at crisis (when egg-laying was about to stop) it served as a stimulus with the result that the oviposition period was prolonged to 8 days and the number of eggs on an average increased to 151·3. This shows that the normal quota of eggs increased by 98·04%, and the oviposition period was practically doubled.

Evidently, besides the increase in the number of eggs the span of life of the adults also increased considerably.

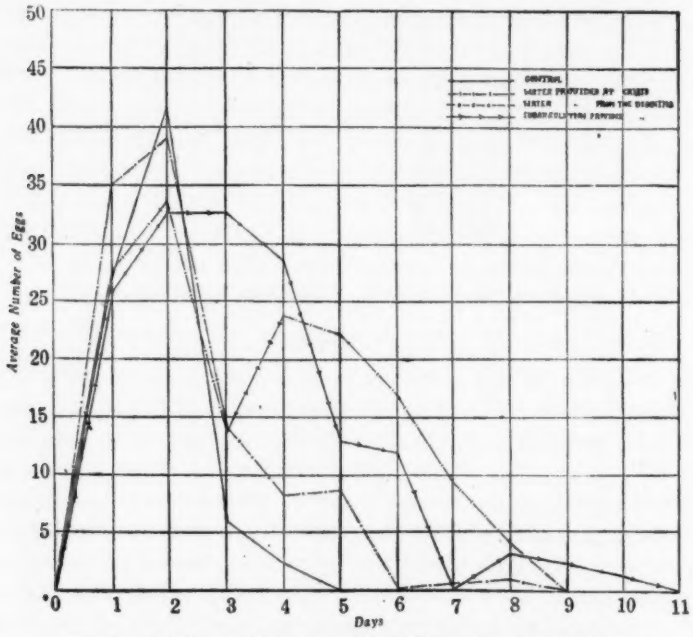

FIG. 3. Relative Oviposition under Different Food Conditions

(*b*) When water was supplied from the beginning the number of eggs in the first two days increased, but ultimately the average number of eggs was only 106·3 being 38·2% greater than in the absence of water. The maximum life of a female, however, extended to 26 days under these conditions.

(3) *Sugar solution.*—Sugar solution from the beginning resulted in increasing the total number of eggs by 98·4% of the normal and the oviposition period by about 4 days. The maximum number of eggs laid by a single female under these conditions was 213 which was the highest recorded so far. The average life of the female under these conditions was 10·3 days against 12·9 days in the male. This was contrary to our previous observations since in most of the cases female, as a rule, outlived the male.

Effect of volatile substances.—

With a view to determining the effect of some vapours on the multiplication of this insect, the following observations were made:—

(*a*) *Effect on oviposition* (Fig. 4).—Newly formed pupæ were exposed to various volatile substances in petri dishes for different durations and the

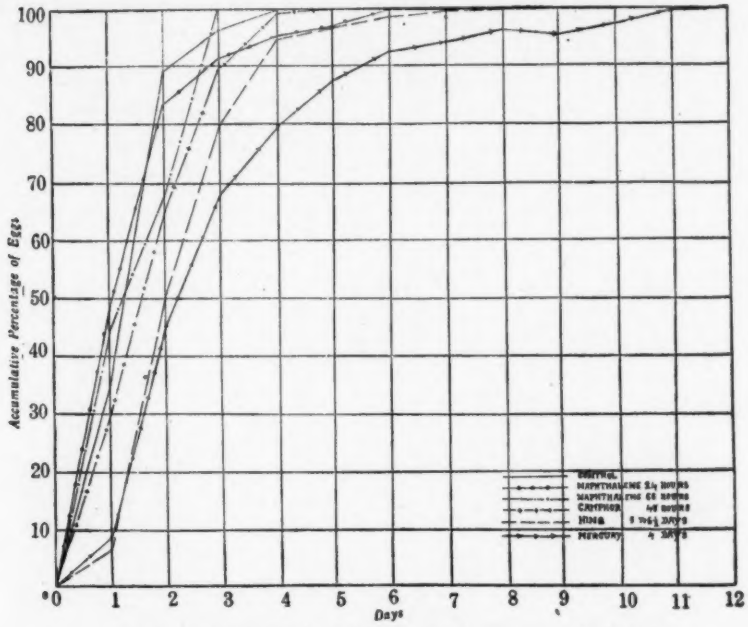

FIG. 4. Relative Effect of Various Substances on Oviposition

adults emerged from them were allowed to oviposit (Tables I and II). Naphthalene produced marked effect in so far as it reduced the oviposition considerably and its effect was progressive till the number of eggs was reduced by 43·3% under an exposure of 66 hours. The span of life was affected adversely whereas, the oviposition period was narrowed down by one day under the extreme condition. Since the span of life on an average, was relatively longer than under normal conditions, it is presumed that the adverse effect was probably on the functioning of the gonads and not on other activities. Mercury as well, reduced the number of eggs by 29·7% of the normal although the duration of oviposition was the longest. Camphor and garlic were responsible for lowering the number of eggs by 26·5 and 21·3% respectively. Tobacco smoke and *hing*, however, did not produce any marked effects. As stated previously, the maximum performance of egg-laying was observed on the 2nd day after the commencement of laying and this behaviour remained almost unaffected under various treatments.

TABLE I

Relative oviposition of the potato tuber moth, 1943

Conditions under which oviposition is observed		Duration of oviposition after the emergence of the adults (Days)											
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
Control	A	26·9	41·6	6·0	2·4								
	B	26·9	68·5	74·5	76·9								
	C	34·9	89·1	96·8	100·0								
Supplied water at crisis	A	27·5	33·6	13·8	23·7	22·2	16·9	9·3	4·3				
	B	27·5	61·1	74·9	98·6	120·8	137·7	147·0	151·3				
	C	18·1	40·3	49·5	65·1	79·8	91·0	97·1	100·0				
Provided water from the beginning ..	A	35·0	39·1	14·1	8·2	8·6	0·1	0·3	0·9				
	B	35·0	74·1	88·2	96·4	105·0	105·1	105·4	106·3				
	C	32·9	69·7	82·9	90·6	98·7	98·8	99·1	100·0				
Supplying sugar solution from the beginning	A	25·8	32·8	32·9	28·6	13·0	12·0	0·1	3·4	2·4	1·3		
	B	25·8	58·6	91·5	120·1	133·1	145·1	145·2	148·6	151·0	152·3		
	C	16·9	38·4	60·0	78·8	87·3	95·2	95·3	97·4	99·1	100·0		
Pupæ with naphthalene for 24 hours ..	A	33·4	24·4	5·8	3·0	0·9	1·9						
	B	33·4	57·8	63·6	66·6	67·5	69·4						
	C	48·1	83·2	91·6	95·9	97·2	100·0						
Pupæ with naphthalene for 48 hours ..	A	22·1	23·3	6·1	4·8	1·9	0·6	1·0	0·6				
	B	22·1	45·4	51·5	56·3	58·2	58·8	59·8	60·4				
	C	36·5	75·1	85·2	93·2	96·3	97·3	99·0	100·0				
Pupæ with naphthalene for 66 hours ..	A	19·3	10·3	14·0									
	B	19·3	29·6	43·6									
	C	44·2	67·8	100·0									
Pupæ in tobacco smoke for 24 hours ..	A	36·9	27·2	7·4	0·6	0·0	5·7	0·4	0·3				
	B	36·9	64·1	71·5	72·1	72·1	77·8	78·2	78·5				
	C	47·0	81·6	91·1	91·8	91·8	99·1	99·6	100·0				

Pupæ in tobacco smoke for 48 hours ..	A	22·4	34·8	10·1	6·7	1·2							
	B	22·4	57·2	67·3	74·0	75·2							
	C	29·9	76·0	89·4	98·3	100·0							
Pupæ with camphor for 48 hours ..	A	16·8	19·6	14·1	5·6	0·4							
	B	16·8	36·4	50·5	56·1	56·5							
	C	29·9	65·1	89·3	99·2	100·0							
Pupæ with garlic for 4 days	A	23·5	20·8	11·2	0·9	0·9	0·1	0·4	1·7	1·0			
	B	23·5	44·3	55·5	56·4	57·3	57·4	57·8	59·5	60·5			
	C	38·8	73·2	91·7	93·2	94·7	94·8	95·5	98·3	100·0			
Pupæ with mercury for 4 days ..	A	4·8	19·6	12·6	6·1	5·2	1·8	0·8	1·2	0·1	0·4	0·9	0·5
	B	4·8	24·4	37·0	43·1	48·3	50·1	50·9	52·1	52·2	52·6	53·5	54·0
	C	8·8	45·3	68·5	79·8	87·5	92·7	94·2	96·4	96·6	97·4	99·1	100·0
Pupæ with *hing* for 5 to 6 days ..	A	5·6	36·8	24·6	12·5	2·0	1·5	0·8	0·2				
	B	5·6	42·4	67·0	79·5	81·5	83·0	83·8	84·0				
	C	6·6	50·2	79·7	94·6	97·0	98·8	99·7	100·0				

Control .. When nothing was provided as food.
Crisis .. A stage when egg-laying was about to stop.
A .. Average number of eggs.
B .. Accumulative totals.
C .. Percentage of the totals.

TABLE II

No. of observations in each case	Conditions under which egg-laying has been observed	Average temperature during the period		Average No. of eggs laid	Max. No. of eggs laid per female	Min. No. of eggs laid per female	Average span of life (Days)		Remarks
		Max.	Min.				Female	Male	
14	Control	91·9	82·5	76·92	110	37	5·79	4·84	
6	Water at crisis	92·6	83·3	151·33	199	94	8·33	8·00	
8	Water from the beginning	95·3	86·1	106·37	187	53	8·50	5·27	Max. span of life observed was 26 days in the case of a female
9	Sugar solution from the beginning ..	94·6	85·5	152·33	213	78	10·33	12·37	Max. span of life observed was 27 days in the case of a male
15	Pupæ with naphthalene for 24 hours ..	84·9	79·4	69·40	138	0	8·86	6·33	
9	Pupæ with naphthalene for 48 hours ..	82 8	78·2	60·42	122	0	10·88	6·00	
3	Pupæ with naphthalene for 66 hours ..	83·7	78·6	43·66	56	22	7·00	8·00	
9	Pupæ in tobacco smoke for 24 hours ..	83·4	78·5	78·55	108	63	8·88	7·55	
9	Pupæ in tobacco smoke for 48 hours ..	83·2	78·3	75·22	118	16	9·11	7·55	
7	Pupæ with camphor for 48 hours ..	85·3	79·7	56·57	93	0	11·14	8·33	
13	Pupæ with *hing* for 5 to 6 days ..	80·8	77·4	84·00	121	9	11·76	9·31	
8	Pupæ with garlic for 4 days ..	78·7	75·6	60·50	111	10	15·25	9·62	
16	Pupæ with mercury for 4 days ..	79·1	75·7	54·00	128	0	12·62	6·75	

(*b*) *Effect on hatching and the larvæ.*—Freshly laid eggs were similarly exposed and their percentage of hatching recorded. In the presence of mercury and naphthalene, the eggs did not hatch at all whereas under garlic hatching was cent. per cent. In the presence of *hing*, there was cent. per cent. hatching under ventilated conditions but only 56·2% under air-tight conditions.

Besides, newly hatched larvæ were provided with food and kept in glass jars in the presence of naphthalene and camphor. In both these cases, they died soon after the commencement of the experiment. Whereas, under garlic, *hing*, mercury, etc., quite a large number of them survived and developed.

(*c*) *Effect on emergence.*—Pupæ from which the adults were about to emerge out were exposed to some of the substances in glass tubes. The emergence was recorded but it was observed that the adults on emergence died soon after in the presence of naphthalene, without laying any eggs. In other cases, however, the adults survived and ultimately laid eggs although their performance was affected as stated before.

The emergence was seriously affected under different conditions. Under the influence of naphthalene it was 90·0%, 72·8% and 42·8% when exposed for 24 hours, 48 hours and 75 hours respectively. Exposure under camphor for 48 hours reduced the emergence to 72·9% and the adults died soon after. When pupæ were kept throughout with mercury, *hing* and garlic the emergence on an average, was 93·5%. Under tobacco smoke for 48 hours, the emergence was 68·0%, while under benzene the pupæ failed to emerge after an exposure of 48 hours.

It has been observed that of all the substances under observation, naphthalene yielded encouraging results since the eggs entirely failed to hatch and the adults which emerged died soon after.

Relative infestation under naphthalene treatment.—

Experiment was performed in six glass cells each of which contained 1½ lbs. of perfectly sound potatoes. In each of these cells one dozen pupæ of *Phthorimæa operculella* Zell. were introduced. Three of these cells were covered with glass lids to provide almost airtight conditions and the other three had wire-gauze lids for aeration. In two of the cells with glass lids half a ball of naphthalene was introduced while in two others with wire-gauze lids, one ball each was placed. Other two cells—one with glass lid and the 2nd with wire-gauze lid, were kept as control. Observations were made after a fortnight. In cells with naphthalene under ventilated conditions, the

potatoes were absolutely sound. In the other lot with glass lids, they were not affected by the pest at all, but some of them had developed mould and were smelling of naphthalene. In the control cages, however, the infestation was 66·6% under airtight conditions and cent. per cent. under ventilated conditions (Table III).

TABLE III

Development under Naphthalene and Mercury Treatment

Sl. No.	Weight of potatoes (lbs.)	Nature of the lid	Quantity of Naphthalene	No. of pupæ	No. of pupæ failed to emerge	Condition of potatoes after 15 days
1	1½	Wire-gauze	1 ball	12	2	Quite good, unaffected by the pest
2	1½	do.	do.	12	4	do.
3	1½	Glass	½ ball	12	3	Potatoes attacked by mould. Slightly smelling of naphthalene, accumulation of water on the sides of cell.
4	1½	do.	do.	12	6	do.
5	1½	Wire-gauze	Control	12	4	100% infestation. Badly attacked
6	1½	Glass	do.	12	6	66·6% infestation

Note.—The percentage of infestation has been calculated irrespective of the number of adults emerged.

Similar observations were made with caterpillars under naphthalene and mercury vapours. One pound of sound potatoes was stored in each of the 8 glass cells—four with glass lids and the others with wire-gauze lids. In each of these cells 75 newly hatched larvæ were introduced. After one week when the larvæ had practically established in the tubers, a drop of mercury was introduced in four cells—two with glass lids and two with wire-gauze. In the 2nd set of four, ¼th of naphthalene ball was introduced. From one week after this treatment, observations were started and the number of dead larvæ, pupæ and adults were recorded. The observations were continued till emergence stopped altogether. In cells containing naphthalene, no pupation or emergence was noticed while in those containing mercury one adult emerged even under airtight conditions while under ventilated conditions 28·6% pupated and 5·3% emerged as adults.

Summary

The life-history and bionomics of *Phthorimæa operculella* Zell. has been studied under Poona conditions. Effect of various volatile substances has

been worked out. Naphthalene has yielded very encouraging results in so far as oviposition may be reduced by about 43·3% if the pupæ are exposed to its vapour for about 66 hours. The eggs do not hatch at all and the adults which emerge under these conditions die soon after without laying. The infestation even when present in the tubers does not develop further. The effect is equally beneficial in airtight and ventilated conditions. Other substances have not yielded very encouraging reasults.

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REGENERATION OF GRASSES IN USAR SOILS

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(With one Plate)

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1. Introduction

Two types of alkali soils are found in India, usar and reh. While usar soils are hard and compact, reh soils are soft, powdery and white. Both these types extend from the Punjab to Bengal in Northern India and in certain parts of Southern India. Voelcker (1898) had estimated that in the North-West Frontier Province alone there were between four to five thousand square miles of usar land. In the Deccan and Southern Mahratta country, too, are similar large tracts of usar soils. In 1883 the total area of the usar land in the United Provinces was computed as 3,129,053 acres. This figure included alkali and saline areas. According to a statement prepared by the Board of Revenue (1939) the total area of usar and reh lands now stands at a lower figure than before, *viz.*, 2,037,119 acres.

It has long been known that the alkali lands have great intrinsic fertility. But in order to utilize them it is necessary to free them of their noxious salts.

Attempts are, therefore, being made in the United Provinces as in other countries to reclaim them and make them fit for the growth of crop plants. Dhar (1935) has given a brief history of alkali soils and their reclamation. In India such attempts have been made by several workers. Leather (1914) tried various methods of reclamation and found that usar can be reclaimed best by the application of gypsum as advocated by Hilgard (1905). But it proved to be uneconomical due to excessive cost of the chemical. Dalip Singh and Nijhawan (1932)* who treated the alkali soils with a mixture of calcium chloride and calcium sulphate reported an increase of permeability after four years, the addition of calcium salts being continued every year. The hydrogen-ion-concentration came down from 9·2 to 8·2 after treatment.

Dhar (1935) claims that for reclamation of usar soils of the dry tracts of Northern India, molasses which is one of the by-products of sugar industry can be used very beneficially. According to him molasses being acidic helps to neutralize the sodium carbonate of the alkali soils. It also increases the soil micro-organisms, the humus, the nitrogen and the ammonia contents. Experiments carried out by Dhar at Allahabad show that the moisture content of the soil treated with molasses is appreciably higher than the untreated soil. Molasses to the extent of 100–500 maunds per acre when employed by him in some of the alkaline fields in the United Provinces produced a good crop of sunn hemp after two years of application.

The permanent reclamation of usar soils which is inseparably connected with the genetics of soil concerned is, however, a difficult task and will take long to achieve. Efforts to reclaim usar soils should, therefore, be supplemented by attempts at its utilization by growing indigenous plants of economic value. Such attempts are already being made in other countries.

One way of utilizing the usar soils is to grow indigenous fodder grasses of high nutritive value for grazing purposes particularly as there is a great dearth of pasture land in these provinces and in other parts of India.

With that end in view attempts were made to survey the number of indigenous grasses (fodder and non-fodder) already present in usar soil and to study the efficacy of simple means of enclosure and ploughing on the regeneration of grasses in usar soils. The results are given in the present paper.

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* Original not seen.

2. *Method*

In order to find out the number of species of grasses growing in usar soils and the extent of regeneration shown by these underploughed and unploughed conditions when protected from grazing and grass cutting the following methods were employed:—

A typical usar area was marked out into sub-plots. Each of these sub-plots was ploughed by a different type of tractor leaving the rest unploughed. The area was then enclosed by barbed wire fencing to allow undisturbed growth of plants in the differently ploughed and unploughed areas.

For comparison of the grasses growing outside where the grazing was freely occurring with those growing inside in the ploughed and unploughed sub-plots an extensive collection was made during different times of the year and identified.

A quantitative study of the grasses growing in the unenclosed area, in ploughed and unploughed plots, was made by quadrat methods.

Each quadrat measured one metre square and it was marked by four nails at the four corners with strong iron wire running along the sides of the square. This was then subdivided into smaller squares to facilitate counting of different species. Such quadrats were laid on the unploughed, the differently ploughed areas and the meadow outside the enclosure. All the species found within the quadrat were charted and counted. Further details of the methods followed for these and other experiments are given at their appropriate places.

The Experimental Plot.—The plot of land available for the experiment is situated about a mile west of the ninth mile Cawnpore road from Lucknow. The plot is an enclosed area of 136 acres in the midst of a vast meadow of typical unculturable usarl and (Plate III, Fig. 1). Portions of the plot were ploughed by different tractors in December 1936 and the entire plot was fenced in August 1937 to protect it from grazing and grass cutting (Plate III, Fig. 2). A portion of the experimental plot is shown in Figs. 5 and 6, Plate III. It was available to the author in September 1938 for a period three years.

The enclosed area is not level throughout. The land situated in the north-east corner is lower where water accumulates during the rainy season and soil condition there is different from the rest of the area. There is another low-lying area near the middle of the plot which shows similar waterlogging condition during the rainy season with a consequent difference

in the soil character. The rest of the experimental plot shows more or less uniformity in its level.

Ploughing.—The plot had been ploughed by different tractors in December 1936. Each tractor worked on a sub-plot of 500′ × 800′ with strips of land of about the same dimensions separating the areas ploughed by them.

The different tractors employed along with their horse power, the area tilled by each, the depth of ploughing, and the quality of work turned out by each of them are shown in Table I.

TABLE I

Table showing the particulars of the tractors employed and their performances

Name of the tractor	Area ploughed in acres	H.P. of the tractor at the draw bar	Average depth ploughed in inches	Quality of work turned out
Hanomag	9·18	42·15	8·25	Even ploughing
Caterpillar	9·18	45·38	10·75	do.
Gyrotiller (small) ..	4·51	40·00	9·00	Ploughed land full of clods, thorough disintegration claimed
do. (big) ..	9·18	180·00	11·75	do.
Fordson	3·75	11·00	6·00	Even ploughing
McCormick	7·08	44·66	9·00	Uneven ploughing due to clutch stoppages

It will be seen from the above table that the average depth for ploughing varies from 6″ by Fordson to 11·75″ by Gyrotiller (big). Hanomag ploughed to the depth of 8·25″, Gyrotiller (small) and McCormick both ploughed 9″ deep and Caterpillar 10·75 inches.

The quality of work turned out was also different. McCormick and Gyrotiller (big and small) produced clods of various sizes whereas the other tractors, *viz.*, Hanomag, Caterpillar and Fordson ploughed evenly.

In all the cases in the course of ploughing two actions, (i) tilling the soil and (ii) inverting the soil, were performed simultaneously.

The draw bars of the tractors penetrated to a certain depth and then threw the soil upside down. The furrows formed by tilling the soil in one row were filled up by the soil of the second row. The result was that contrary to the working of the country plough, the ploughing by all these tractors was done uniformly without forming any furrows.

3. Types and Distribution of Soils

A survey of the soil conditions of the experimental plot in the ploughed and in the unploughed areas showed the presence of both usar and reh soil.

The usar soil is more extensive both in the unploughed and differently ploughed sub-plots. Reh soil on the other hand is restricted only to the ploughed areas and is distributed in smaller or larger scattered patches. (Plate III, Figs. 3 and 4). It does not, however, occur in the unploughed plots except where the soil has been undisturbed. The extent of land under usar and reh in the differently ploughed sub-plots is given in Table II.

TABLE II

Table showing the areas of Usar and Reh soils in the differently ploughed sub-plots

Name of the tractor	Total area ploughed in sq. ft.	Area under usar soil in sq. ft.	Area under reh soil in sq. ft.	% Usar soil	% Reh soil
Hanomag	4,00,000	3,07,040	92,960	76·8	23·2
Caterpillar	4,00,000	3,35,630	64,370	83·9	16·1
Gyrotiller (small)	1,96,456	1,72,856	23,600	87·9	12·1
do. (big)	4,00,000	3,87,600	12,400	96·9	3·1
Fordson	1,63,350	1,54,950	8,400	94·8	5·2
McCormick	3,08,404	2,91,504	16,900	94·5	5·5
TOTAL ..	18,68,210	16,49,580	2,18,630	89·1	10·9

The above table shows that along with usar soil which is in great majority, reh soil is found in all the ploughed areas. Total area under reh is about 11%, the rest being usar. The extent of reh in differently ploughed areas varies a good deal. In the sub-plots ploughed by Hanomag, Caterpillar, Gyrotiller (small and big), Fordson and McCormick the reh produced is 23·2%, 16·1%, 12·1%, 3·1%, 5·2% and 5·5% respectively. It is very uncertain, however, that there is any relation between the tractors used and the extent of reh that is produced. But it seems fairly certain that under the conditions of experiment reh is produced by the disturbance of the soil due to ploughing.

4. Effect of Ploughing on the Regeneration of Grasses in an Enclosed Area

For this exepriment the species of grasses growing in the ploughed and unploughed enclosed areas were collected and identified. The results are given in Table III.

TABLE III

Table showing the different species of grasses and sedges in the enclosed ploughed and unploughed areas

Sl. No.	Name of species	Ploughed enclosed area	Unploughed enclosed area
1	*Aristida adscensionis* Linn.	+	+
2	*Bothriochloa pertusa* A. Camus	+	+
3	*Chloris montana* Roxb.	+	+
4	*Chloris virgata* Sw.	+	+
5	*Cynodon dactylon* Pers.	+	+
6	*Cyperus bulbosus* Vahl.	−	+
7	*Cyperus diformis* Linn.	+	+
8	*Cyperus iria* Linn.	+	+
9	*Cyperus procerus* Rottb.	+	+
10	*Cyperus rotundus* Linn.	+	+
11	*Dactyloctenium ægyptium* Beauv.	+	+
12	*Dichanthium annulatum* Stapf.	+	+
13	*Echinochloa colonum* Link.	+	+
14	*Eragrostis coarcata* Stapf.	+	+
15	*Eragrostis gangetica* Steud.	+	+
16	*Eragrostis pilosa* Beauv.	+	+
17	*Eragrostis tenella* Roem et Schult.	+	+
18	*Eriochloa procera* C. E. Hubbard	+	+
19	*Fimbristylis schoenoides* Vahl.	+	+
20	*Ischæmum rugosum* Salish.	+	+
21	*Iseilema laxum* Hack.	+	+
22	*Panicum humile* Nees.	+	+
23	*Panicum psilopodium* Trin.	+	+
24	*Panicum trypheron* Schlut.	+	+
25	*Paspalidium flavidum* A. Camus.	+	−
26	*Paspalum scrobiculatum* Linn.	+	+
27	*Setaria glauca* Beauv.	+	−
28	*Sporobolus arabicus* Boiss.	+	+
29	*Sporobolus coromandelianus* Kunth.	+	−
30	*Sporobolus glaucifolius* Hochst.	+	−

It will be seen from Table III that altogether 30 different species of grasses and sedges were found in the ploughed and the unploughed areas. The ploughed areas had 29 species and the unploughed areas had 26 species. 25 species were common to both the areas. *Cyperus bulbosus* was found in the unploughed area but it was absent from the ploughed areas. On the other hand *Paspalidium flavidum, Setaria glauca, Sporobolus coromandelianus,* and *S. glaucifolius* were present only on the ploughed areas.

Since the ploughing was done in 1936 and all the existing grasses had been uprooted in the process, the grasses in the ploughed areas have established themselves within three years. The origin, development, and establishment of these grasses which have occurred subsequent to ploughing must have started either from seeds and uprooted root stock or from the seeds of grasses migrated from the adjoining undisturbed soil with the result

that within these three years as many as 29 species have established themselves on the denuded areas.

With a view to find out the frequency of various plants occurring inside and outside the enclosure, a quantitative study of the flora was made by means of quadrats. The quadrats were laid on the representative parts of the ploughed sub-plots wherein all 18 quadrats were laid out of which 12 were in Hanomag ploughed sub-plot, 4 in Caterpillar, and 2 in the Gyrotiller (small) ploughed sub-plot. All the different species of grasses occurring within these quadrats along with the number of individual plants of each of those species were counted and charted on graph papers. The results obtained are given below in Table IV in which the dominant and subsidiary grasses in various quadrats are given in third and fifth columns respectively along with the number of individuals of each given in 4th and 6th columns.

TABLE IV

Table showing the relative concentration of grasses in each quadrat of the variously ploughed sub-plots

Name of plough	No. of quadrats	Dominant species	No. of individuals	Sub-dominant species	No. of individuals
Hanomag	1	*C. montana*	711	..	..
	2	*do.*	617	..	..
	3	*do.*	440	*Dactyloctenium ægyptium*	37
				S. arabicus	31
				Paspalidium flavidum	29
	4	*do.*	435	..	..
	5	*do.*	397	..	..
	6	*do.*	393	..	..
	7	*do.*	384	*Dactyloctenium ægyptium*	7
	8	*do.*	320	..	..
	9	*do.*	320	..	..
	10	*do.*	223	*S. arabicus*	39
				S. coromandelianus	193
	11	*S. arabicus*	297	*C. montana*	263
	12	*Dactyloctenium ægyptium*	378	*do.*	193
Caterpillar	1	*C. montana*	433	..	..
	2	*do.*	427	*S. arabicus*	25
	3	*do.*	328	..	..
	4	*do.*	297	*S. arabicus*	223
Gyrotiller (small) ..	1	*do.*	130	*Panicum* sp.	4
	2	*do.*	95	*S. arabicus*	73

It will be seen from Table IV that in the Hanomag ploughed area *C. montana* is the dominant species in 10 out of 12 quadrats. In the two instances where *S. arabicus* and *Dactyloctenium ægyptium* are dominant, *C. montana* is co-dominant in one having 263 individuals against 297 of *S. arabicus* and sub-dominant in the other having 193 individuals against 378 of *Dactyloctenium ægyptium.*

The results find confirmation from the data obtained from the Caterpillar and Gyrotiller small ploughed sub-plots where, in all the 6 quadrats *C. montana* was the dominant species.

The numerical strength of *S. arabicus* and *C. montana* as found from the quadrat study on the unploughed and the differently ploughed sub-plots is given in Table V.

TABLE V

Table showing the numerical strength of S. arabicus *and* C. montana

Plot	No. of quadrats	Total No. of individuals		Average per quadrat	
		C. montana	*S. arabicus*	*C. montana*	*S. arabicus*
Ploughed	18	6,406	688	356	38
Unploughed	19	563	5,499	29	289

From Table V it will be seen that numerical strength of *C. montana* and *S. arabicus* in the unploughed area is 29 and 289 individuals per quadrat respectively. The number of individuals of *C. montana,* however, increases to 356 per quadrat and those of *S. arabicus* decreases to 38 per quadrat in the ploughed land.

The results show that *C. montana* propagates and regenerates more rapidly in the enclosed and ploughed areas than in the unploughed areas and that *S. arabicus* propagates more rapidly in enclosed unploughed areas.

5. Regeneration of Grasses on Reh Soils in the Ploughed Enclosed Area

The species growing on reh soils and their density were ascertained by using the same methods as were employed for usar soils. Only 4 species of grasses were found growing in the reh soil. These grasses are very sparsely distributed but major part of the reh is absolutely barren. Without a single grass, in other places only isolated individuals are seen. In certain areas, particularly those nearer to the unploughed area, the number of

individuals is greater. From a few quadrat studies of areas of average density it was found that *S. coromandelinus* and *S. glucifolius* have only 2 and 6 individuals per quadrat, whereas *C. montana* and *S. arabicus* show 94 and 60 individuals respectively. The two are co-dominant. A similar area in usar soil will contain about 350 individuals of *C. montana* and 40 of *S. arabicus*. It is apparent, however, that colonization has started in reh soil but the process is very slow.

6. Effect of Enclosure on the Regeneration of Grasses in Unploughed Soil

In open meadow the plants are subjected to intensely close grazing and are thus unable to retain their normal size and fail to flower and seed. The vegetative growth, even where it is a normal mode of propagation, is checked. Due to indiscriminate grazing many of the grasses and annuals disappear. Protection of an area produces a condition in which plants can grow undisturbed.

A comparison of the species of grasses growing under unploughed enclosed and unenclosed areas shows that the species of grasses growing outside the enclosure are much less in number than those inside the enclosure. Thus there are 26 species in the unploughed enclosed area as against 6 in the unploughed meadow outside it. The grasses growing on the unenclosed meadow which could be identified are: *Chloris montana, Chloris virgata, Setaria glauca, Sporobolus arabicus, S. coromandelianus* and *S. glaucifolius*. Of the 26 species found inside the enclosure there are five species belonging to the family Cyperaceæ.

A quantitative study of the flora was made by means of quadrat method with a view to find out the frequency of various plants occurring inside and outside the enclosure. The results obtained are given in Table VI, where the species and number of individuals of each species growing in each quadrat are given separately. Wherever there are more than one species given for a quadrat the first named species is dominant as can be seen from the number placed against it.

It is seen from Table VI that in unploughed enclosed areas in 16 out of 19 quadrats *S. arabicus* is dominant. In one quadrat it is subdominant with *C. montana* as the dominant species. In unploughed unenclosed area *C. montana* is dominant in certain cases and *S. arabicus* in others.

The numerical strength of *C. montana* and *S. arabicus* as found from the quadrat study on the unploughed enclosed area and the unploughed meadow is given in Table VII.

TABLE VI

Table showing the relative concentration of grasses in each quadrat of the unploughed enclosed area and the meadow outside it

Place	No. of quadrats	Dominant species	No. of individual plants	Subsidiary species	No. of individual plants
Unploughed enclosed ..	1	*S. arabicus*	632	..	..
	2	*do.*	481	..	..
	3	*do.*	427	..	..
	4	*do.*	413	..	..
	5	*do.*	387	..	..
	6	*do.*	381	..	..
	7	*do.*	370	..	..
	8	*do.*	345	..	..
	9	*do.*	332	..	..
	10	*do.*	311	*C. montana*	61
	11	*do.*	297	*do.*	139
	12	*do.*	227	*do.*	9
	13	*do.*	199	..	..
	14	*do.*	198	*C. montana*	25
	15	*do.*	167	..	..
	16	*do.*	157	*Paspalidium flavidum*	116
	17	*C. montana*	257	*S. arabicus*	175
	18	*Echinochloa colonum*	359	*C. montana*	72
	19	*Cyperus rotundus*	287	..	..
Unploughed unenclosed ..	1	*C. montana*	123	*S. arabicus*	6
	2	*do.*	102	*do.*	59
	3	*S. arabicus*	93	*C. montana*	67
	4	*do.*	63	*do.*	5

TABLE VII

Table showing the numerical strength of C. montana *and* S. arabicus *on the unploughed enclosed and unenclosed areas*

Place	No. of quadrats	No. of individuals		Average per quadrat	
		C. montana	*S. arabicus*	*C. montana*	*S. arabicus*
Unploughed enclosed ..	19	563	5,499	29	289
Unploughed unenclosed ..	4	297	221	74	55

From Table VII it will be seen that so far as *S. arabicus* is concerned the largest number, average 289 individuals per quadrat, is found in unploughed protected area as against 55 in the unploughed meadow outside. It shows clearly that *S. arabicus* propagates in unploughed areas when

protected from grazing and grass cutting, all other conditions being the same. The unploughed enclosed land, however, shows a decrease in the number of *C. montana* which is 29 as against 74 in the meadow outside it. In outside meadow average strength of *C. montana* is 74 as against 55 of *S. arabicus*. Grazing, therefore, seems to affect *S. arabicus* more than *C. montana.*

The plants growing inside the enclosure showed a marked gain in the size, increase in the percentage of seedlings produced and an increase in the number of spikelets in each plant and thus an all-round increase in the seed production.

Out of 30 species of grasses and sedges growing in the enclosed area, ten species, *viz.. Paspalidium flavidum, Echinochloa colonum, Dichanthium annulatum, Iseilema laxum, Cynodon dactylon, Dactyloctenium ægyptium, Setaria glauca, Eragrostis* sp., *Chloris montana* and *Sporobolus arabicus* were good fodder grasses. Most of these species were not found outside the enclosure but it is possible that these were either completely eradicated or if present could not be identified due to the close and preferential grazing. All these ten species of grasses have established themselves on the experimental plot within a period of three years. Their presence there suggests that the simple process of enclosure will allow some of the fodder grasses to grow and establish themselves on such soils. It is, therefore, suggested that some of the grasses of the first category should be tried for introduction in usar soil.

7. *Discussion*

The process of regeneration is constantly going on among the grasses growing in usar soils. In the unenclosed usar meadow where grazing, grass cutting and other interfering factors are constantly at work regeneration cannot take place. The grasses are unable to attain normal size; they do not flower and reproduction through seeds, therefore, hardly ever occurs. The vegetative propagation takes place only to a limited extent.

In the foregoing pages are given the data for the regeneration of grasses in the unploughed and variously ploughed areas under enclosure. In the unploughed areas the soil is entirely undisturbed. Before enclosing the land the cattle had grazed freely and grass cutting there had also been unrestricted. Prior to fencing the unploughed enclosed area was no different from the unenclosed, unploughed and closely grazed meadow outside. It is, therefore, expected that whatever changes have occurred on the unploughed enclosed area within these three years are due to enclosure alone.

Making allowance for the difficulties in identifying some of the grasses due to their close grazing by cattle on the meadow, a large number of grasses make their appearance under protected condition. Of these *S. arabicus* is by far the dominant species with about 5,500 individuals in 19 one metre quadrat followed by *Chloris montana* with 560 individuals in the same number of quadrats. So far as the investigation goes *S. arabicus* shows a greater capacity to dominate over *C. montana* and other grasses when protected from grazing and grass cutting. The position is somewhat different in the meadow outside where from the results obtained from the limited number of quadrats it can be deduced that *C. montana* is dominant over *S. arabicus*.

Ploughing had changed the soil character. During the course of ploughing the inverted soil brought up the salts present in the lower horizon. The soil character underwent further alteration due to weathering and leaching effects of the monsoon rains of 1937, 1938 and 1939.

The ploughed areas entirely denuded of the vegetation have the most suitable soil for invasion, migration and colonization. It is here that regeneration of grasses can be studied in full details.

The ploughing was done in 1936 winter and all the species of grasses then growing had been uprooted. In the ploughed areas, therefore, whatever regeneration of grasses occurred was due to some of the root stocks that held on to the soil and to seeds which were present in the soil.

After the enclosure, during 1937 monsoon the grasses protected from grazing had an uninterrupted growth. In this year too there were those grasses in the denuded areas as were already present in the soil along with such grasses growing from seeds and root stocks of previous season.

In 1938 conditions for the regeneration of grasses became more suitable. The grasses that regenerated and had attained maturity in 1937, seeded, and the seeds dispersed on the denuded soil, the soil condition due to leaching was less extreme, hence a large number of individuals appeared occupying extended areas.

In 1939 under still favourable conditions with a larger number of species inhabiting the soil the advance of the grasses was considerably greater than in the previous years.

In the course of three years' protection from grazing therefore, there have appeared 30 different grasses and sedges. These have originated from the seeds and root stocks present in the soil or from seeds migrated from neighbouring areas.

The number of individuals counted by means of quadrats revealed the fact that in all differently ploughed sub-plots, namely Hanomag, Caterpillar, Gyrotiller (small and big), Fordson and McCormick, it is *C. montana* which is dominant over species like *Sporobolus arabicus, Eragrostis* sp., *Cynodon dactylon* and *Dactyloctenium ægyptium.*

It is thus observed that *S. arabicus* is the dominant species in the unploughed enclosed area (Plate III, Fig. 8) and sub-dominant in the meadow outside it. *C. montana,* on the other hand, is the dominant grass of the ploughed enclosed areas (Plate III, Fig. 7) as also of the meadow outside the enclosure. Robertson (1937) working in Makhdoompur farm which is about 24 miles away from Lucknow on Cawnpore road found that undergrazing *C. montana* increases and *S. arabicus* decreases. The following table gives the percentages of grasses as found by him in 1936:—

C. montana	*S. arabicus*	*Cyperus* sp. specially *C. iria*	Miscellaneous Species	
			Nutritious	Coarse
34–57%	31–51%	2–9%	4–13%	0–2%

It, therefore, appears that when *S. arabicus* is either partially removed by controlled grazing (Robertson, 1937), or by indiscriminate grazing (meadow), or completely removed by ploughing (present experimental plot), it is always *C. montana* which first encroaches the denuded area and tries to exclude *S. arabicus.* It is only when the land is simply enclosed and not interfered later on that *S. arabicus* remains the chief grass.

The immediate effect of excluding all cattle from an usar area is, therefore, the production of a more luxuriant growth of grasses and their rapid extension over bare areas. The extensive foliage gives a thicker coating over the soil and helps to minimise the scorching rays of the sun and consequently diminishes the upward capillary movement of the salts. It thus prepares suitable ground for other grasses which take advantage of the improved condition of the soil. As conditions improve more valuable grasses like *Iseilema laxum, Andropogon annulatus* and *Cynodon dactylon,* etc., encroach upon such soils whenever it becomes fitted for their existence.

Regeneration of grasses both in the ploughed and the unploughed areas takes place mostly in the usar soil. The reh is also invaded but to a very small extent. Only four species of grasses are found in the reh soil within the enclosed area. The number of individuals too is small. In the case

of the reh soil all the grasses proved to be co-dominant. The extreme soil conditions did not favour the growth of any particular grass growing within the area.

A few of the grasses growing on the experimental plot were first class fodder grasses. These species were not found outside the enclosure. It is possible that these were either completely eradicated or if present could not be identified due to close and preferential grazing. Their presence, however, suggests that the simple process of enclosure will allow the fodder grasses to grow and establish themselves there.

8. Summary

The paper deals with the effect of enclosure on regeneration of grasses in ploughed and unploughed usar soil and the possibility of introducing indigenous grasses capable of establishing themselves on such soils in competition with grasses of less nutritive value.

The investigation was carried out in a typical piece of usar land parts of which were ploughed by different types of tractors leaving the rest unploughed and was subsequently enclosed. It has been found that on usar soil the enclosure considerably helps in the regeneration of grasses. The number of species and the number of individuals of each species are much greater inside the enclosure than in the meadow where the grazing goes on freely. *Chloris montana* is the dominant species in the grazed area whereas in the protected area *Sporobolus arabicus* is dominant. The grazing, therefore, seems to affect *S. arabicus* more than *C. montana.*

Inside the enclosure in unploughed areas *S. arabicus* is the dominant genus with *C. montana* as sub-dominant. In the ploughed area on the other hand *C. montana* is dominant and *S. arabicus* is sub-dominant. It is suggested that *C. montana* is dominant in ploughed area as it regenerates both by root stocks and seeds. *S. arabicus* is dominant in enclosed unploughed areas as it is a perennial grass as against *Chloris montana* which disappears from the soil for about three months during hot weather.

Reh soil is present in ploughed areas alone and covers 11% of the whole experimental plot. It appears that ploughing helps the formation of reh. Reh soil is mostly barren and its colonization is very slow. Only four different species were found growing in reh soil of which *chloris montana* and *sporobolus arabicus* were co-dominant.

Out of thirty species of grasses and sedges found in the usar soil there are ten species which are regarded as fodder grasses of nutritive value. It is suggested that some of these may be tried for introduction in usar soil.

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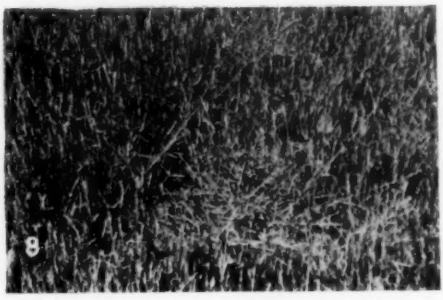
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EXPLANATION OF PLATE

PLATE III

Fig. 1. General view of the large tract of usar land outside the enclosure where a large number of cattle can be seen grazing.

Fig. 2. Photograph showing a portion of the experimental plot along with the meadow outside the enclosure. It will be seen that there is more or less no grass on the meadow outside whereas within the enclosure there is a luxuriant growth.

Fig. 3. Portion of Caterpillar ploughed sub-plot showing *C. montana* growing on usar area whereas reh is lying bare with just a few colonies of grasses.

Fig. 4. An enlarged view of the above.

Fig. 5. In the foreground is the unploughed area with *S. arabicus*. After that the ploughed area has either bare reh or *C. montana* on usar soil. In the background can be seen *Acacia arabica* growing.

Fig. 6. Gyrotiller ploughed area with luxuriant growth of *C. montana*. Some parts have been invaded over by *S. arabicus*.

Fig. 7. *C. montana*, the chief grass of all the ploughed areas.

Fig. 8. *S. arabicus*, the chief grass of all the unploughed areas.

THE INFLUENCE OF HOOK-WORM INFECTION ON THE HEART MUSCLE—AN EXPERIMENTAL INVESTIGATION

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R. E. Heilig and S. K. Visveswar (1942) found that in cases of Ankylostomiasis the heart muscle was damaged; and in 90 per cent. of their cases the cardiac condition improved after iron therapy while in the remaining 10% no improvement was seen until anthelmintic treatment was administered. They suggest that the cardiac muscle in Ankylostomiasis is affected by two factors, the microcytic hypochromic anæmia and a possible toxin. As any toxic effect on the heart muscle has to be exercised *via* the coronary circulation the responsible factor must be present in the blood. It was found desirable to investigate the presence of the toxin experimentally on a suitable cardiac tissue. The perfused isolated frog heart was chosen as the most suitable for this investigation. The experimental procedure was to perfuse the isolated frog hearts with certain known concentrations of blood sera of patients suffering from Ankylostomiasis and to note the results. A certain number of experiments with blood sera of normal individuals was also performed as a control.

Experimental

Cases of Ankylostomiasis with ova positive in the stools, with an anæmia showing 10–20% Hb (Sahli) and with marked cardiac damage were selected from the medical wards of the Krishnarajendra Hospital. The blood was drawn by suitable aseptic technique and collected in a dry test-tube which was given a serial number. The serum was allowed to separate out at laboratory temperature (25°–27° C.) for 24 hours after which it was pipetted out into a dry test-tube. The serum was centrifuged if it was not clear. The test-tube containing serum was stored in the refrigerator until use. The experiments were generally performed on the same day or as soon as possible. Three samples of blood were taken from each patient as follows:—

Sample I	..	Before treatment (ova +, Hb 10–20%).
Sample II	..	After iron therapy (ova +, Hb 35% and above).
Sample III	..	After anthelmintic treatment (ova −, Hb 40% and above).

Medium-sized frogs were used in making the heart preparations by exposing the hearts in pithed frogs and ligaturing all veins except the Inferior Vena Cava into which a suitable cannula was introduced. The heart was then dissected out, the cannula fixed to a stand, and the apex of the heart connected by hooks and thread to a light lever writing on a long paper kymograph. The hearts were perfused with Ringer-Locke solution for frog hearts. The apparatus was so arranged as to allow interchange between normal frog-heart solution and the solution containing known concentrations of serum. Altogether 93 sera were tested including 11 normal sera.

Preliminary experiments showed that any depressant effect on the frog-hearts by perfusing with solutions of ankylostome sera could be obtained only in great dilutions. Concentrations of 1 to 10^4 and above caused a strengthening of beats rather than weakening. The strengthening in higher concentrations was traced to a serum effect because feeding the hearts with undiluted serum (introduced directly into the cannula) caused a great increase in the amplitude of the beats and this effect was obtained equally and constantly both with normal and ankylostome sera. In concentrations ranging from 1 in 10^6 to 1 in 10^5, however, the ankylostome sera produced depression of the heart beats, an effect which was not obtained or obtained only to a slight extent by normal sera in the same strengths. It was observed that in the higher concentrations the depressant effect was masked in the earlier stages of the perfusion by the predominant serum effect, but became apparent later when the serum effect had been overcome.

As a result of the experience gained in the preliminary experiments it was found desirable to try in each case the effect of (1) undiluted serum and (2) serum in different concentrations, the most suitable being 1 in 10^6, 1 in 10^5, and 1 in 10^4. Control experiments were performed with normal serum in the same strengths. As a check on animal variations experiments were performed by frequently repeating the same concentrations and by using concentrations other than those mentioned above. In each case about 100–200 c.c. of serum solution was perfused so as to give sufficient time for the effect to manifest itself demonstrably before changing over to normal solution. Sufficient time was also given for the heart to recover its normal rhythm before starting the next perfusion with serum solution. Toxic effect (T) and Serum effect (S) were designated by single, double or triple + according to magnitude. Negative results were indicated by −.

Fig. 1 illustrates the difference between the effects produced by ankylostome serum (AS/25) and two normal sera (NS/2 and NS/3) when perfused in 1 in 10^5 strength on the same heart preparation. Marked

diminution in the amplitude of the beats is seen in the case of the ankylostome serum.

Fig. 2 illustrates the strengthening of beats produced by 1 in 10^4 solution of ankylostome serum (As/25). This is traced to a serum effect because

TABLE I

Perfusion findings in fourteen cases

No.	Patient	Sample Hb %	1 in 10^6		1 in 10^5		1 in 10^4		Serum	
			T	S	T	S	T	S	T	S
1	K.L.G. ..	I 12	+	−	+	−	+	−	−	++
		II 45					+	−	−	++
		III 45	+	−	−	−	−	−	−	+++
2	M.D.M. ..	I 13	+	−			−	++	−	+++
		II 35	−	−					−	+++
		III 38	−	−					−	+++
3	M.L.G. ..	I 18	+	−					−	++
		II 35	−	−					−	++
		III 40	−	−			−	−	−	++
4	R.M.A. ..	I 18	++	−					−	++
		II 40	+	−					−	+++
		III 50	−	−			−	++	−	+++
5	H.M.G. ..	I 10	++	−					−	++
		II 40	++	−			−	+	−	+++
		III 40	−	−			−	+	−	+++
6	K.C.G. ..	I 12	+	−			+	−	−	++
		II 37	+	−			−	++	−	++
		III 40	−	−	−	−	−	−	−	++
7	V.K.T. ..	I 16	++	−	+	−	−	+	−	+++
		II 38	−	−					−	+++
		III 43	−	−			−	−	−	+++
8	A.D.N. ..	I 13	+	−	+	−	−	+	−	+++
		II 38	−	−	+	−	−	+	−	+++
		III 48	−	−	−	−	−	−	−	++
9	K.L.A. ..	I 10	++	−	+++	−	+	−	−	+++
		II 35	+	−	++	−	+	−	−	+++
		III 40	−	−	+	−	−	−	−	++
10	J.V.R. ..	I 15	+	−	++	−	−	+	−	+++
		II 42	−	−	+	−			−	+++
		III 50	−	−	−	−			−	+++
11	C.N.P. ..	I 11	+	−					−	+++
		II 35	+	−	+	−	−	+	−	+++
		III 45	−	−	−	−	−	+	−	++
12	H.P.G. ..	I 15	+++	−	++	−			−	++
		II 42	+++	−	++	−	++	−	−	+++
		III 60	++	−	+	−	−	+	−	+++
13	P.T.I. ..	I 10	+	−	+	−	−	+	−	++
		II 40	−	−	−	−	−	+	−	++
		III 40	−	−	−	−	−	+	−	++
14	K.R.Y. ..	I 15	++	−	+	−	−	+	−	++
		II 45	+	−	+	−	−	−	−	++
		III 50	−	−	−	−	−	+	−	++

N.B.—Blanks indicate that no experiments were performed.

undiluted serum (both ankylostome As/25 and normal NS/2) produces the same effect in a greater degree.

Figs. 3 and 4 illustrate the differences in the effects produced by Sample I (before treatment), Sample II (after iron therapy), and Sample III (after anthelmintic treatment). Toxic effect is seen with Sample I in strengths of 1 in 10^6 and 1 in 10^5. In the same strengths Sample II shows persistence of the toxic effect in one case (Fig. 4) and absence of the effect in the other case (Fig. 3). Sample III shows absence of the toxic effect in both cases (Figs. 3 and 4).

Table I gives the results of perfusion experiments in 14 cases. Serum toxæmia (T) of varying degree is present in strengths of 1 in 10^6 and 1 in 10^5 with respect of Sample I in all cases. The toxæmia persists in 8 cases with respect of Sample II and is present (but diminished in magnitude) in 2 cases with respect of Sample III. Serum effect (S) is negative in all cases in strengths of 1 in 10^6 and 1 in 10^5. It is present to a slight degree in some cases in 1 in 10^4 strength but is markedly present with undiluted serum in all cases.

Table II gives a comparison of perfusion and cardiographic findings with respect of the 14 cases, included in Table I. There is agreement in respect of 8 cases.

TABLE II

Comparison of perfusion and cardiographic results in 14 *cases*

No.	Patients	Perfusion			Cardiogram		
		I	II	III	I	II	III
1	K.L.G.	+	+	+	±	⊙	⊙
2	M.D.M.	+	−	−	+	> >	> >
3	M.L.G.	+	−	−	++	> >	> >
4	R.M.A.	++	+	−	++	>	>
5	H.M.G.	++	++	−	+	> >	> >
6	K.C.G.	+	−	−	++		> >
7	V.K.T.	++	−	−	+	>	>
8	A.D.N.	+	−	−	++	> >	> >
9	K.L.A.	++	+	−	+		±
10	J.V.R.	+	−	−	+	⊙	⊙
11	C.N.P.	+	+	−	+	>	> −
12	H.P.G.	+++	++	++	++	>	> >
13	P.T.I.	+	−	−	+	> >	> >
14	K.R.Y.	++	+	−	±	>	> >

N.B.—Perfusion :
+ indicates toxic effect
− „ no toxic effect

Cardiogram :
+ indicates heart damage
⊙ „ no improvement
> „ improvement

Table III gives the percentages of positive toxæmia in all the sera examined in respect of Samples I, II and III in strengths of 1 in 10^6 and 1 in 10^5.

TABLE III

Percentages of toxæmia in all sera examined

	Sample		
	I (Untreated) %	II (After iron therapy) %	III (After deworming) %
Hæmoglobin	10–20	35–45	40–60
Serum toxæmia —			
1 in 10^6	100	53	14
1 in 10^5	89	83	25

The following conclusions can be drawn from a study of the experimental results:—

(1) Sera of patients suffering from Ankylostomiasis produce a toxic effect on frog hearts in concentrations of 1 in 10^6 and 1 in 10^5.

(2) Sera of the same patients after iron therapy show the toxic effect only in 53% of cases in 1 in 10^6 strength. In 1 in 10^5 strength however the effect persists in 93% of cases.

(3) Sera taken after anthelmintic treatment show that the effect persists in only 14% of cases in 1 in 10^6 strength and in 25% of cases in 1 in 10^5 strength. Even in the persisting cases the effect considerably diminished.

Discussion

The experimental results indicate that there is some factor present in the blood serum of patients suffering from hook-worm disease which causes a depressant effect on heart muscle and that this factor disappears in a small percentage of cases after iron therapy, but disappears completely or is considerably diminished after anthelmintic treatment. While pointing out the existence of a depressant factor in the serum of the patients our experiments do not warrant any conclusion as to the nature of the factor in question. Reference to literature does not throw any direct light on the influence of hook-worm infection on the heart muscle although the causation of the anæmia has been discussed by various workers. A. C. Chandler (1929) cites three possible ways in which hook-worms may cause anæmia: (1) by direct ingestion of blood from the intestinal walls and hæmorrhage from the abandoned bites, (2) by hæmolysis of the corpuscles in the circulation by absorption of hæmolytic or other destructive substances, and (3) by a toxic

effect on the blood-forming organs. Discussing these factors and the evidence offered by the work of DeLangen (1924) and others, Chandler says that chronic loss of blood is at most a minor factor in hook-worm disease and cannot account for the severe anæmia. Secondly the blood serum of hook-worm patients has low bilirubin value in all stages of the disease and there is also a low excretion of bilirubin, facts indicating absence of a hæmolysin in the circulation. Further, the blood picture gives the impression of unhealthy regeneration rather than chronic loss. Chandler therefore concludes that there is little ground for doubting that the anæmia is due primarily to a toxic effect on the blood-forming organs by a toxin secreted by the worms and thrown into the circulation or by absorption of bacterial toxins, decomposition products of necrotic tissue, or other poisonous substances through the lesions in the intestinal walls. L. E. Napier, C. R. Das Gupta, and D. N. Majumdar (1941) discount the toxæmia theory of the causation of hook-worm anæmia for two reasons, the absence of any evidence of aplasia of the bone-marrow and the absence of any reticulocyte response when the worms are removed. They, however, admit that there is some evidence of a retarding effect of the hook-worm infection on the response to iron therapy and this could be hardly accounted for by simple blood loss. They consider this factor as of minor importance and explainable by failure of absorption as a result of intestinal mucosal dysfunction caused by the presence of the worms. In the light of our experimental results we are inclined to support the toxæmia theory of Chandler. Whether this plays a major role as suggested by Chandler or is only of minor importance as indicated by Napier *et al.* we are not at present in a position to discuss. Our experiments indicate that the blood serum of patients suffering from Ankylostomiasis contains a toxic factor and we feel justified in assuming tentatively that this might be responsible for the effects on the heart as well as on the blood-forming organs. It is proposed to work out the problem in greater detail in another series of experiments on frog hearts and obtain confirmation on mammalian hearts.

It is now necessary to explain the effects seen after iron therapy alone without anthelmintic treatment. On comparing the results of our perfusion experiments in 14 completed cases with the corresponding Cardiographic results of Heilig and Visveswar a certain degree of agreement is seen between the perfusion and cardiographic findings, but obviously no exact correlation could be obtained on account of the inherent differences in the two methods of evaluation. While cardiograms show improvement in 90% of cases after iron therapy, perfusion experiments show disappearance of the toxic factor in about 27% of cases only. All workers agree that complete improvement

of patients in hook-worm disease cannot occur until effective deworming by anthelmintic has been carried out. It is possible that the apparent improvement in the heart condition after iron therapy only is due to the conditions becoming less favourable for the action of the toxin. If no anthelmintic treatment is administered the toxæmia reasserts itself and causes a progressive deterioration in both the cardiac and anæmic conditions.

Our thanks are due to Dr. Robert Heilig for his co-operation in the investigation and for furnishing the cardiographic results. We thank Mr. S. K. Visveswar for supplying the blood samples, hæmoglobin values and other clinical data.

Summary

1. The influence of hook-worm infection on the heart muscle is investigated by perfusing isolated frog hearts with different concentrations of blood sera of patients suffering from Ankylostomiasis.

2. It is observed that in concentrations of 1 in 10^6 and 1 in 10^5 the sera produce a diminution of the Amplitude of the beats indicating the presence of a toxic factor in the sera.

3. The toxic effect disappears in a few cases after iron therapy and disappears completely or is considerably diminished after anthelmintic treatment.

4. It is concluded subject to further experimentation that some factor yet to be isolated is present in the blood serum in Ankylostomiasis which exercises a toxic effect on the cardiac muscle and on the blood-forming organs.

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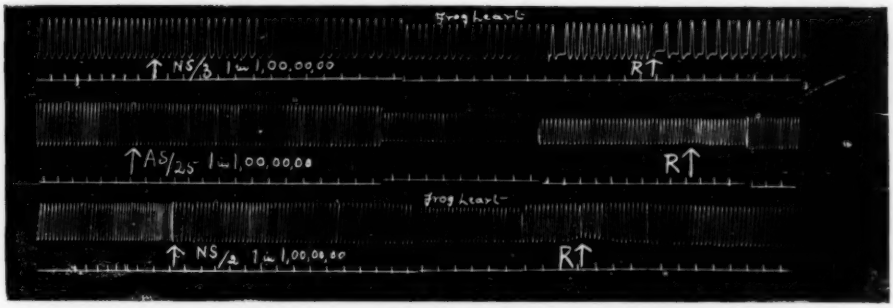

FIG. 1. *Isolated Frog-heart.*—Shows comparative effect of perfusing with ankylostomes serum (AS/25) and two normal sera (NS/2. NS/3) in 1 in million dilutions. There is marked diminution of amplitude in the case of the ankylostome serum while the effect is nil or only slight in the case of the normal sera. All the effects are demonstrated on the same heart preparation (Time = 5 sec., R = Ringer solution).

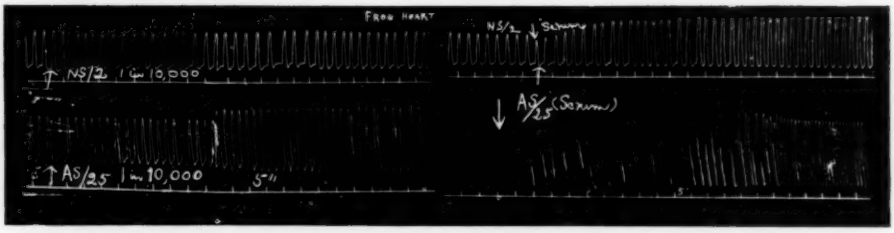

FIG. 2. *Isolated Frog-heart.*—Shows the serum effect of higher concentrations. The increase in amplitude got by perfusing with 1 in 10,000 solution of ankylostome serum (AS/25) is similar to the effect produced by undiluted serum of the same patient (AS/25), and is also got by normal serum NS/2 (Time = 2 sec., R = Ringer solution).

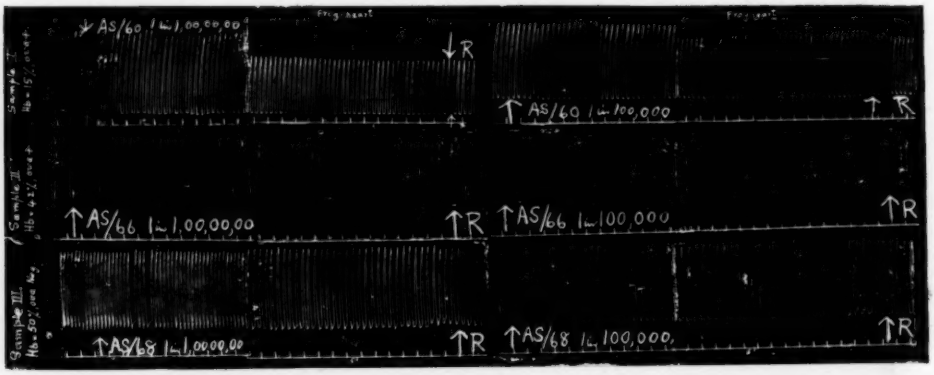

FIG. 3. *Isolated Frog-heart.*—Shows comparison in dilutions of 1 in million and 1 in 100,000 of Sample I (AS/60, untreated case, Hb 15%, ova +ve), Sample II (AS/66, after iron therapy, Hb 40%, ova +ve) and Sample III (AS/68, after deworming. Hb 50%, ova −ve) the blood samples being taken from the same patient. The depressant effect on the heart is seen with Sample I and is absent in Samples II and III (Time = 5 sec., R = Ringer solution).

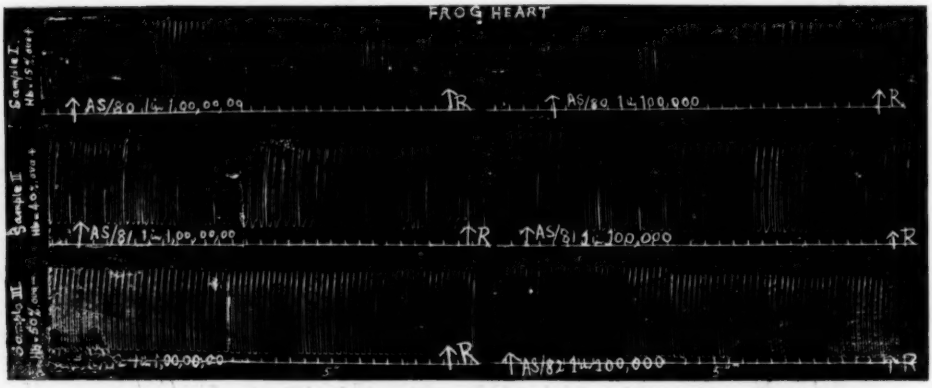

FIG. 4. *Isolated Frog-heart.*—Shows comparison in dilutions of 1 in million and 1 in 100,000 of Sample I (AS/80), Sample II (AS/81) and Sample III (AS/82) from another patient. The depressant effect is present in Samples I and II and is absent in Sample III (Time = 5 sec., R = Ringer solution).

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